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Sakai et al.

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(54) **APPARATUS FOR CAPTURING CELL**

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C12M 3/00 (2006.01)

C12M 1/00 (2006.01)

(52) **U.S. Cl.** **435/285.1**; 435/285.2; 435/287.1; 435/288.5; 435/288.7; 435/297.2; 359/398; 204/403.01

(58) **Field of Classification Search** 435/285.1, 435/288.5, 288.4, 285.2, 287.1, 288.7, 297.2, 435/297.5; 359/398; 204/403.01

See application file for complete search history.

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(57) **ABSTRACT**

A bottom wall of a Petri dish used for capturing a cell is provided with a first through hole and a second through hole. A groove that connects the first through hole and the second through hole is formed on an outer surface of bottom wall. The first through hole, the second through hole, and the groove is covered with a transparent plate member. A plate used for capturing the cell is arranged in the Petri dish above the first through hole. An aspiration tube is connected to the second through hole from inside the Petri dish.

10 Claims, 12 Drawing Sheets

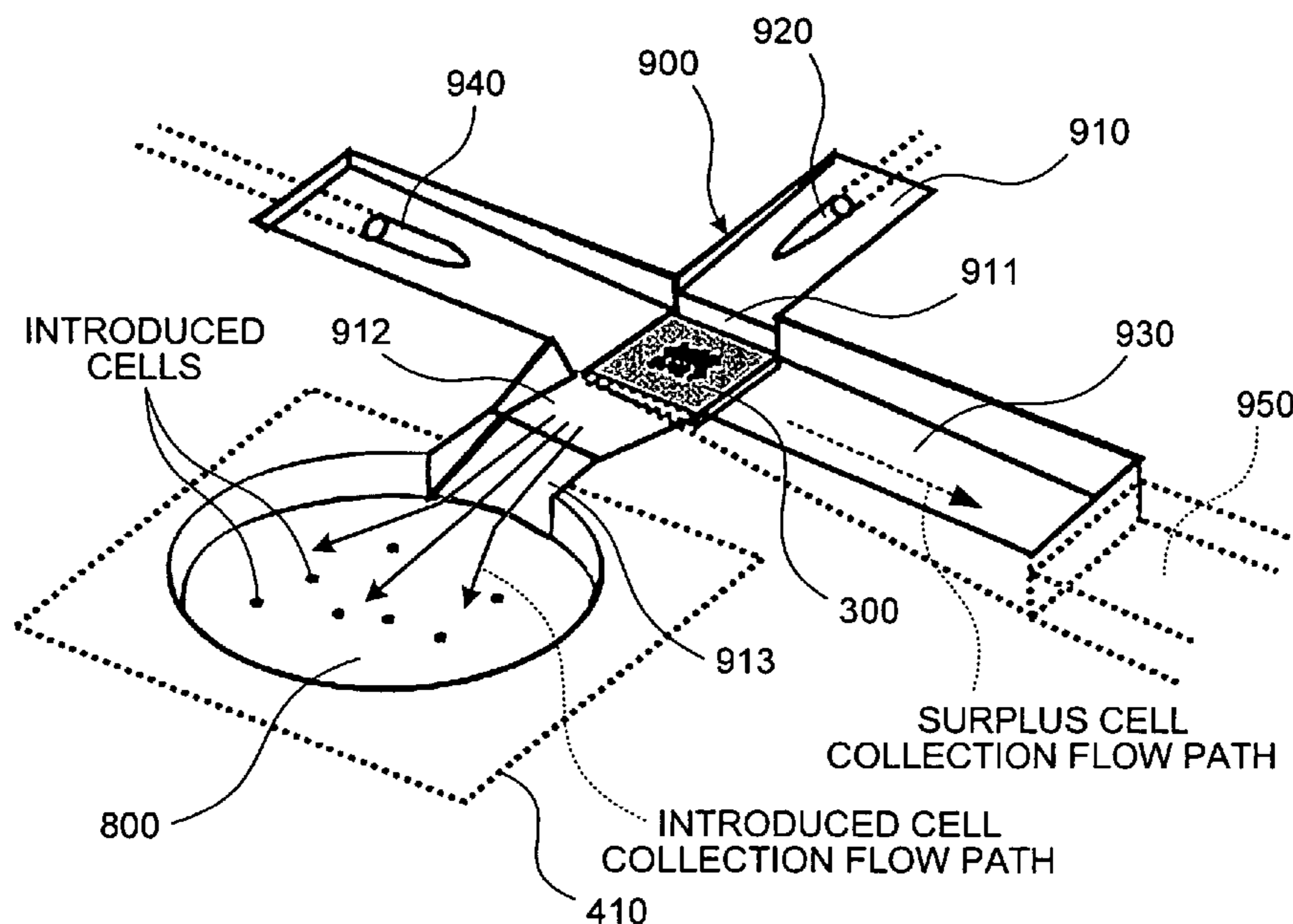


FIG. 1

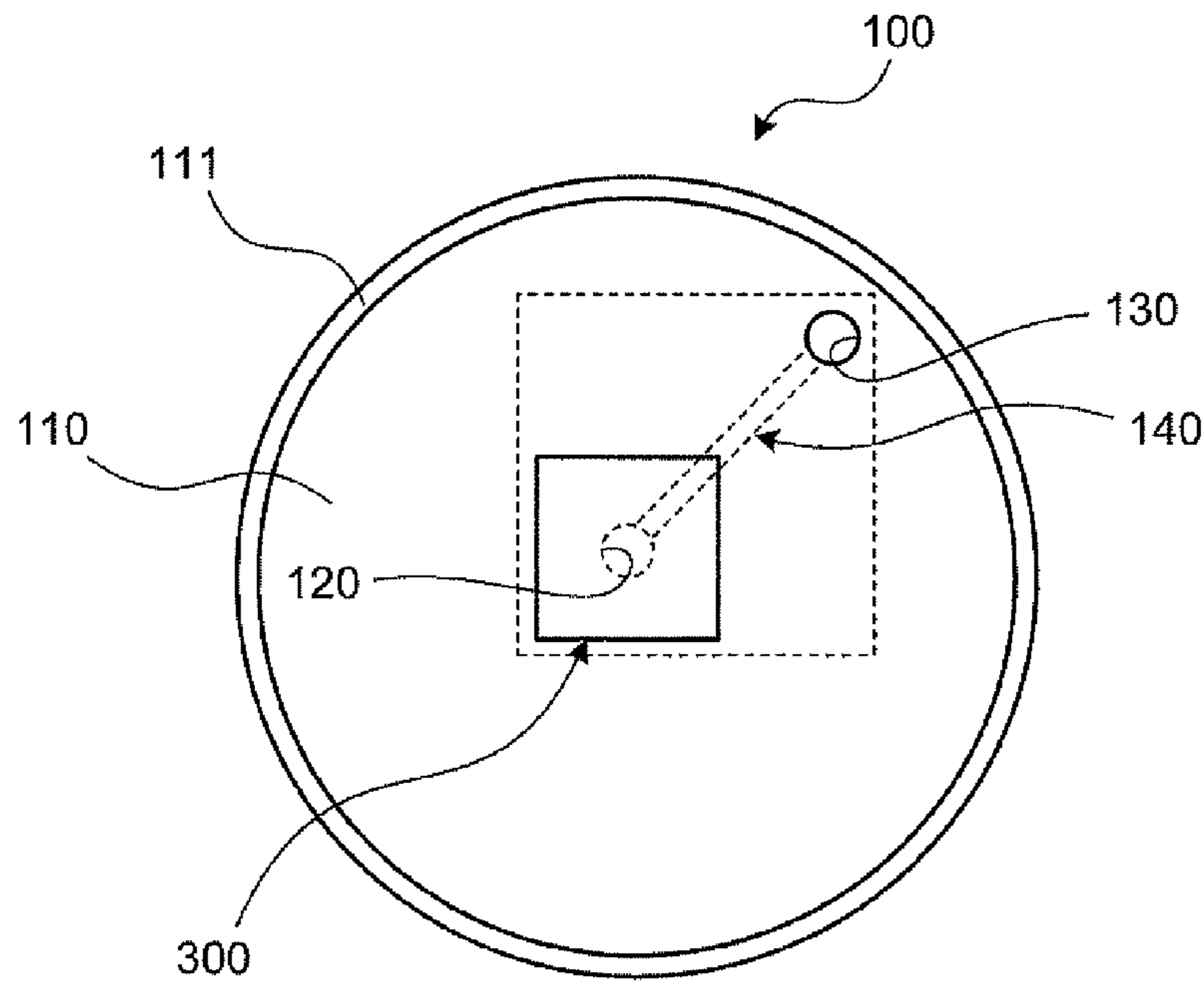


FIG. 2

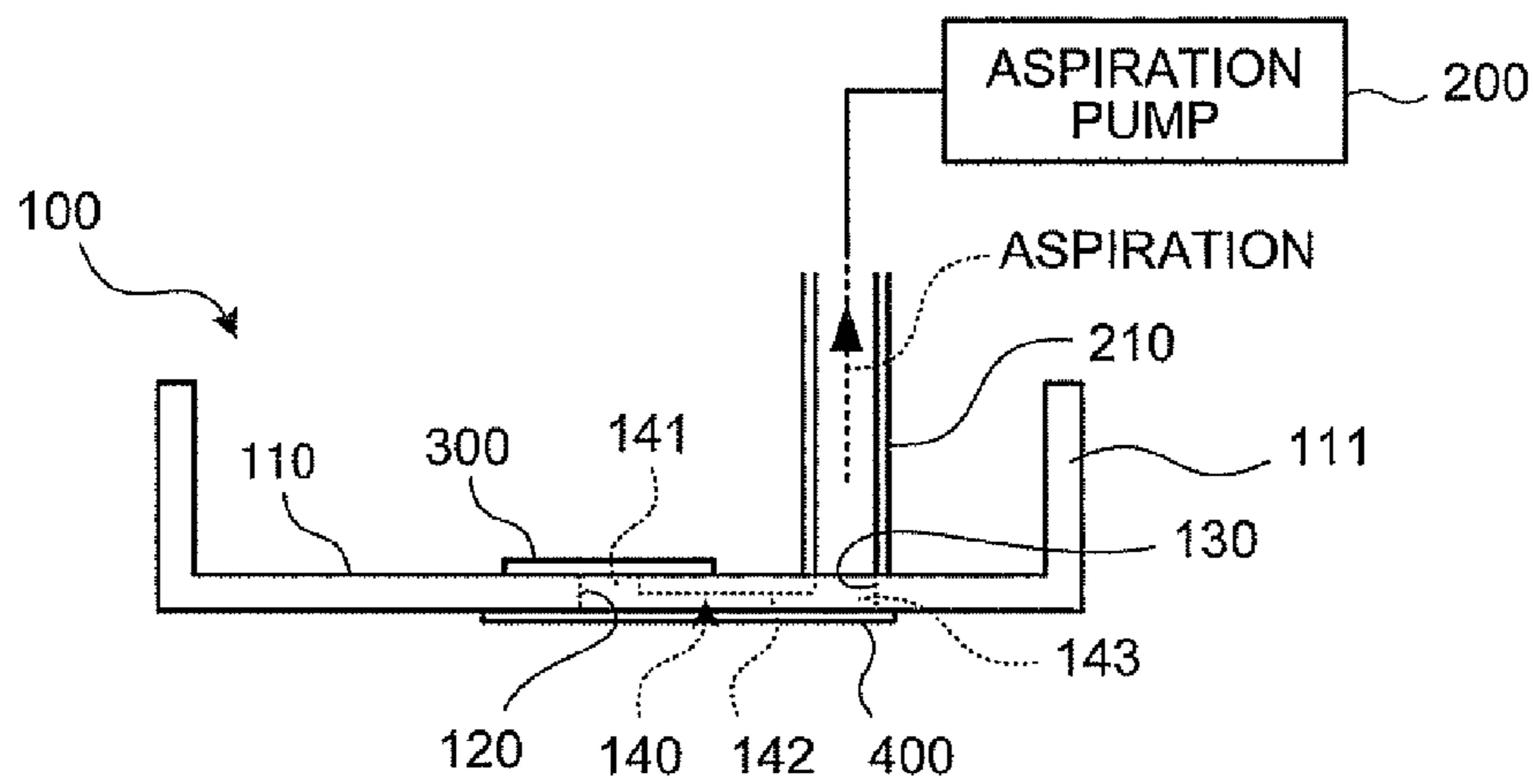


FIG.3

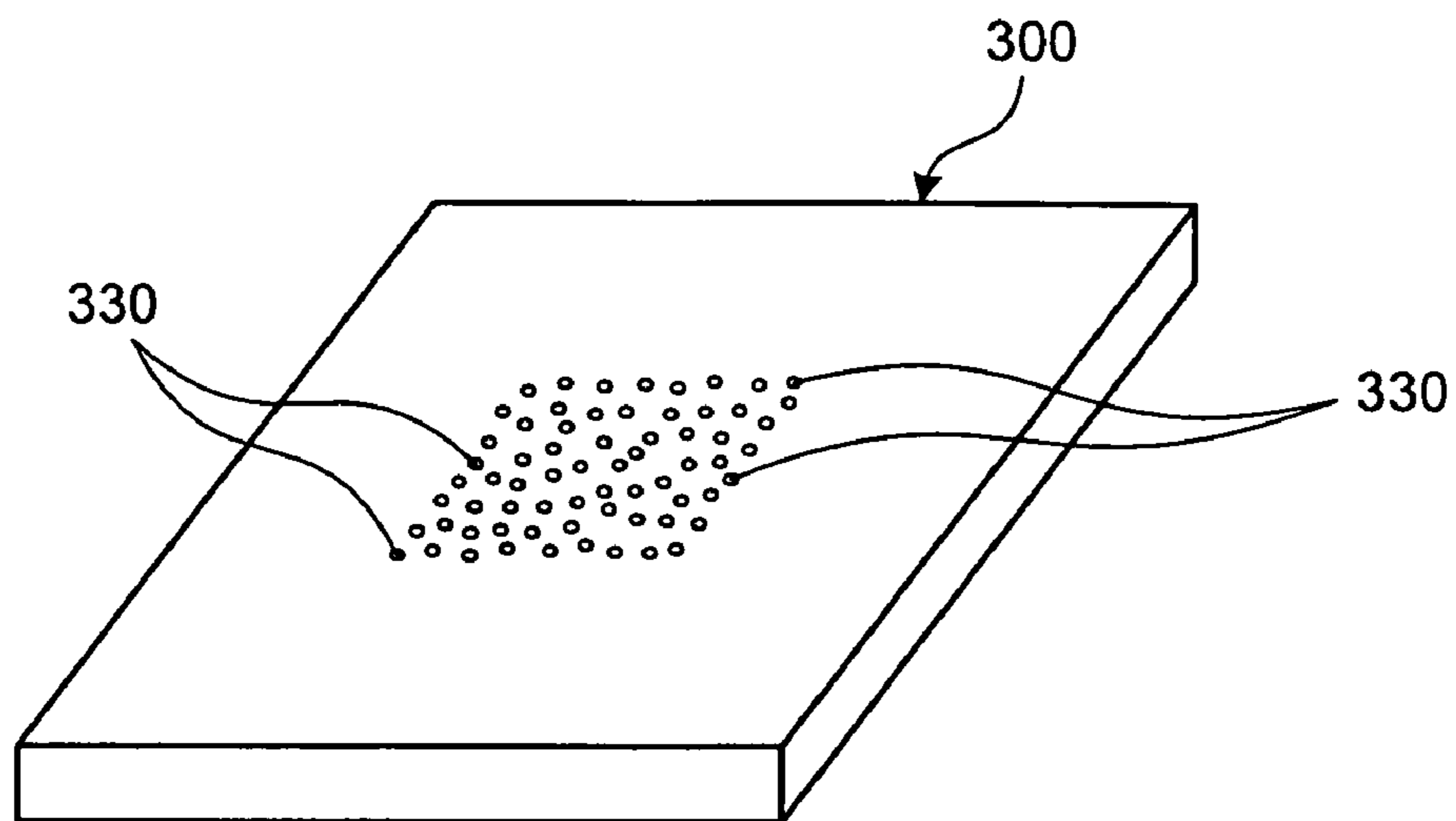


FIG.4

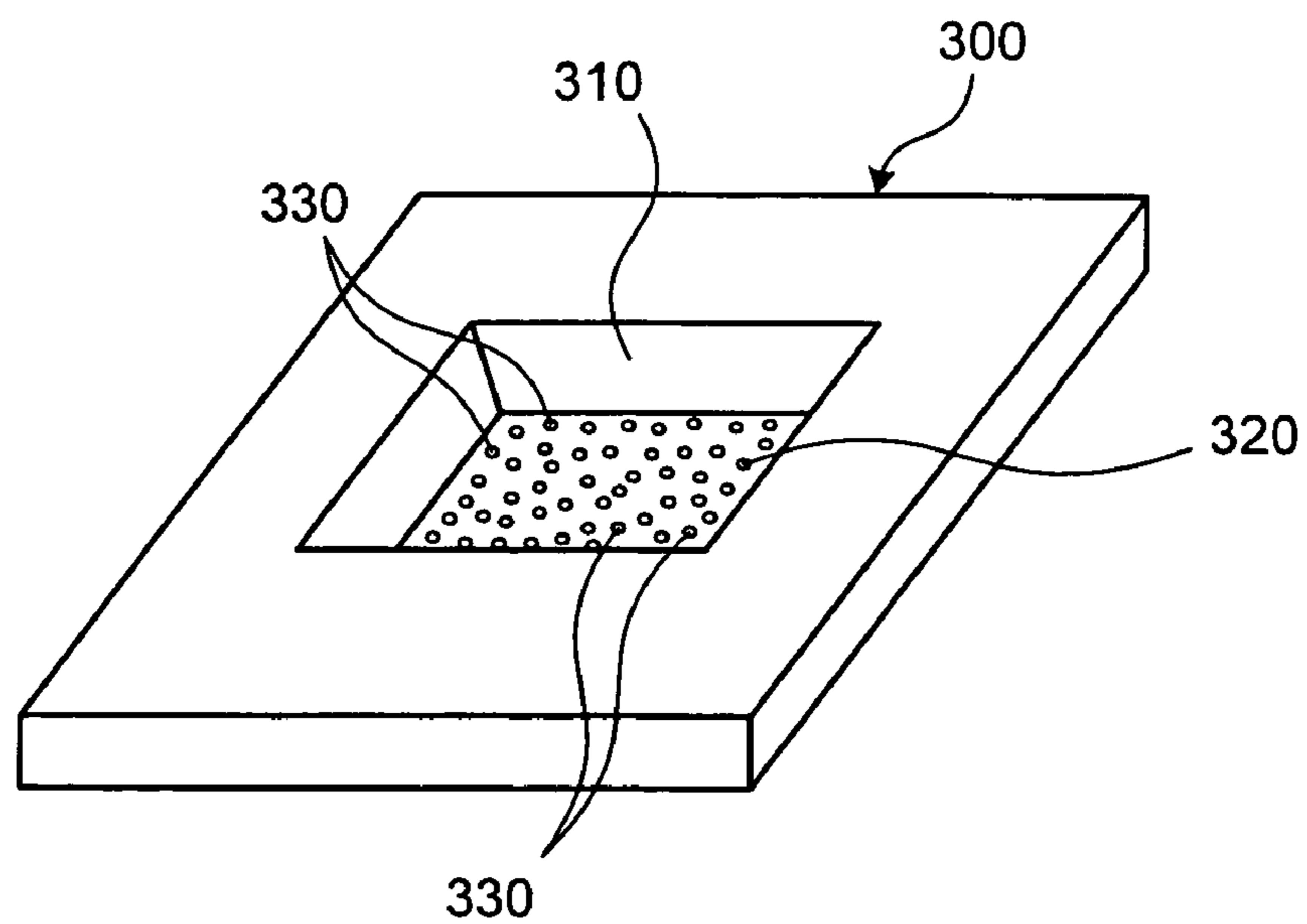


FIG.5A

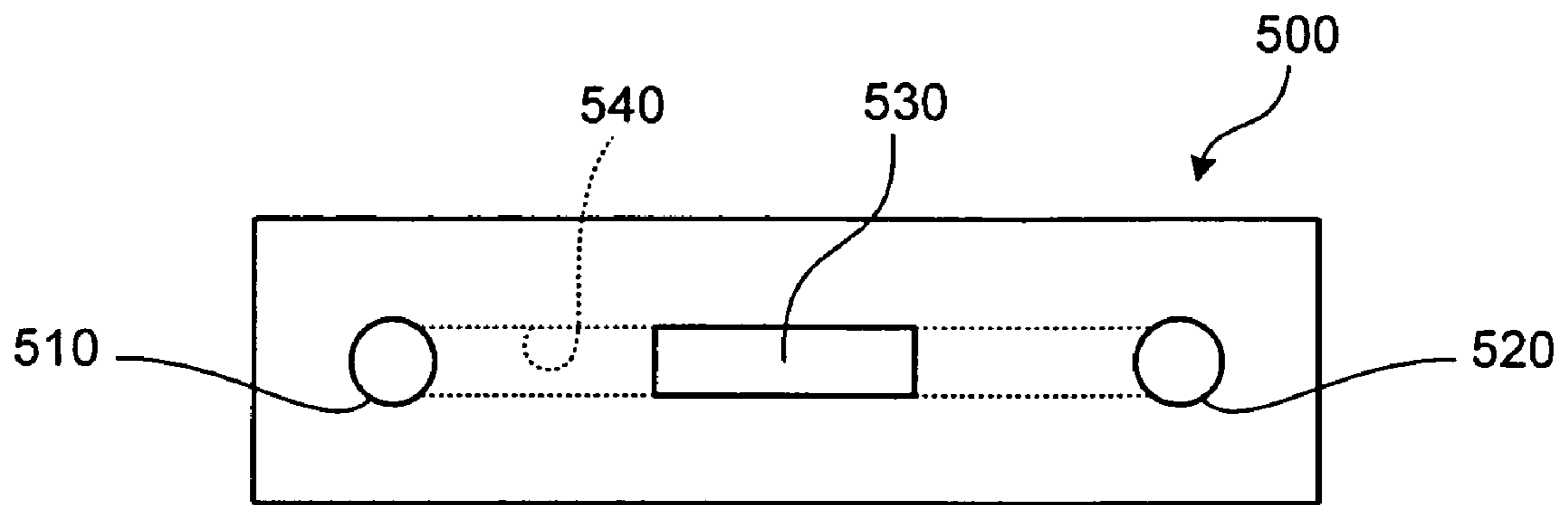


FIG.5B

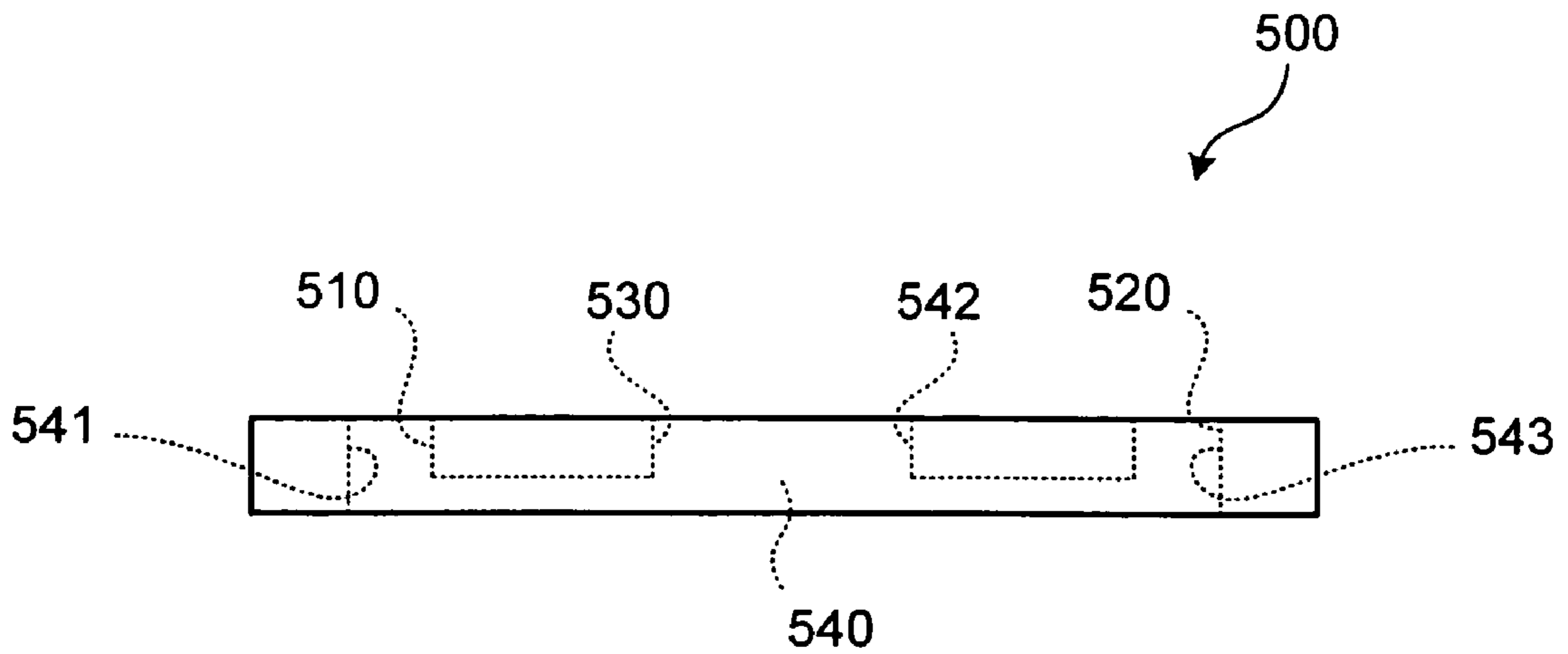


FIG. 6

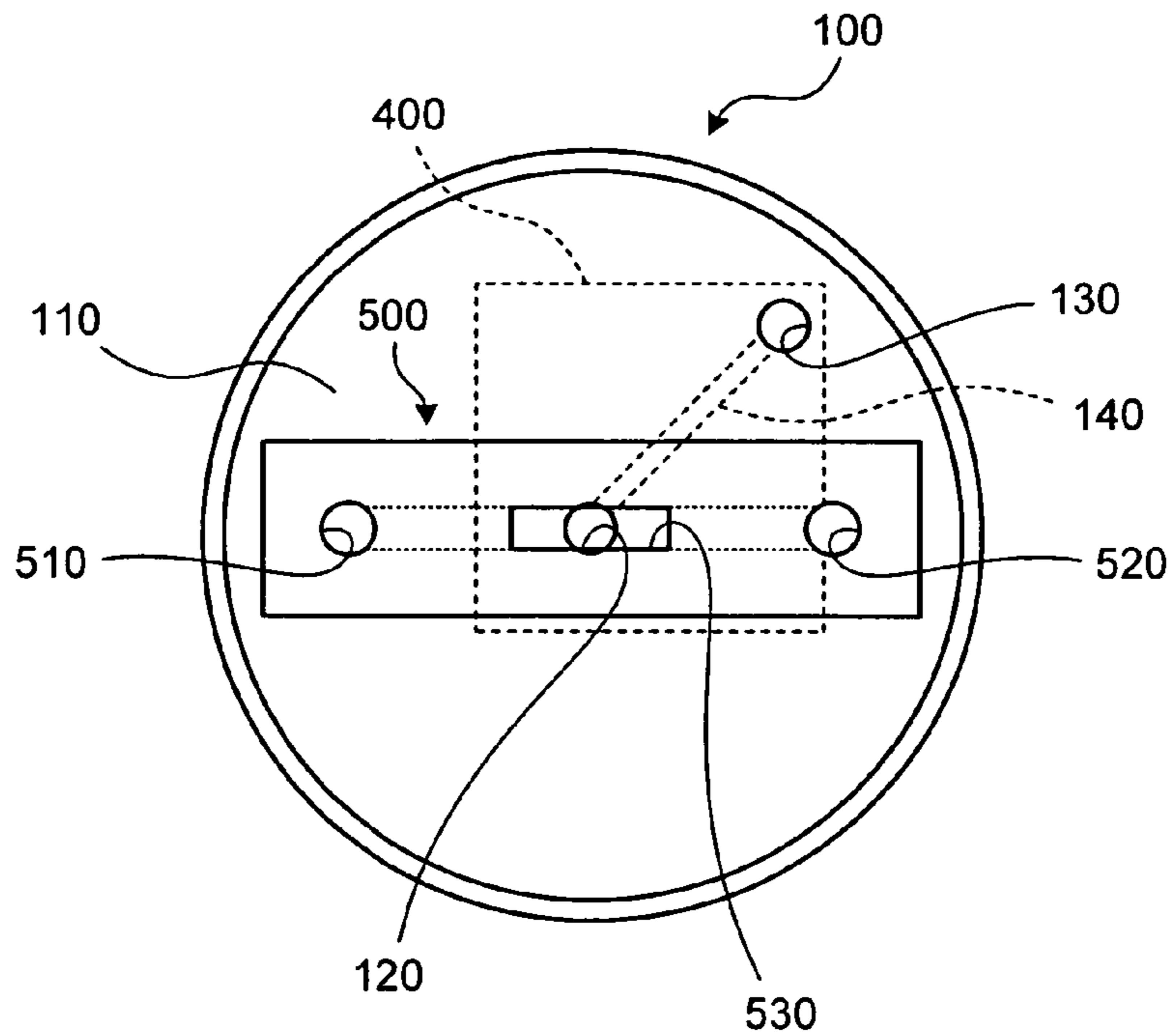


FIG. 7

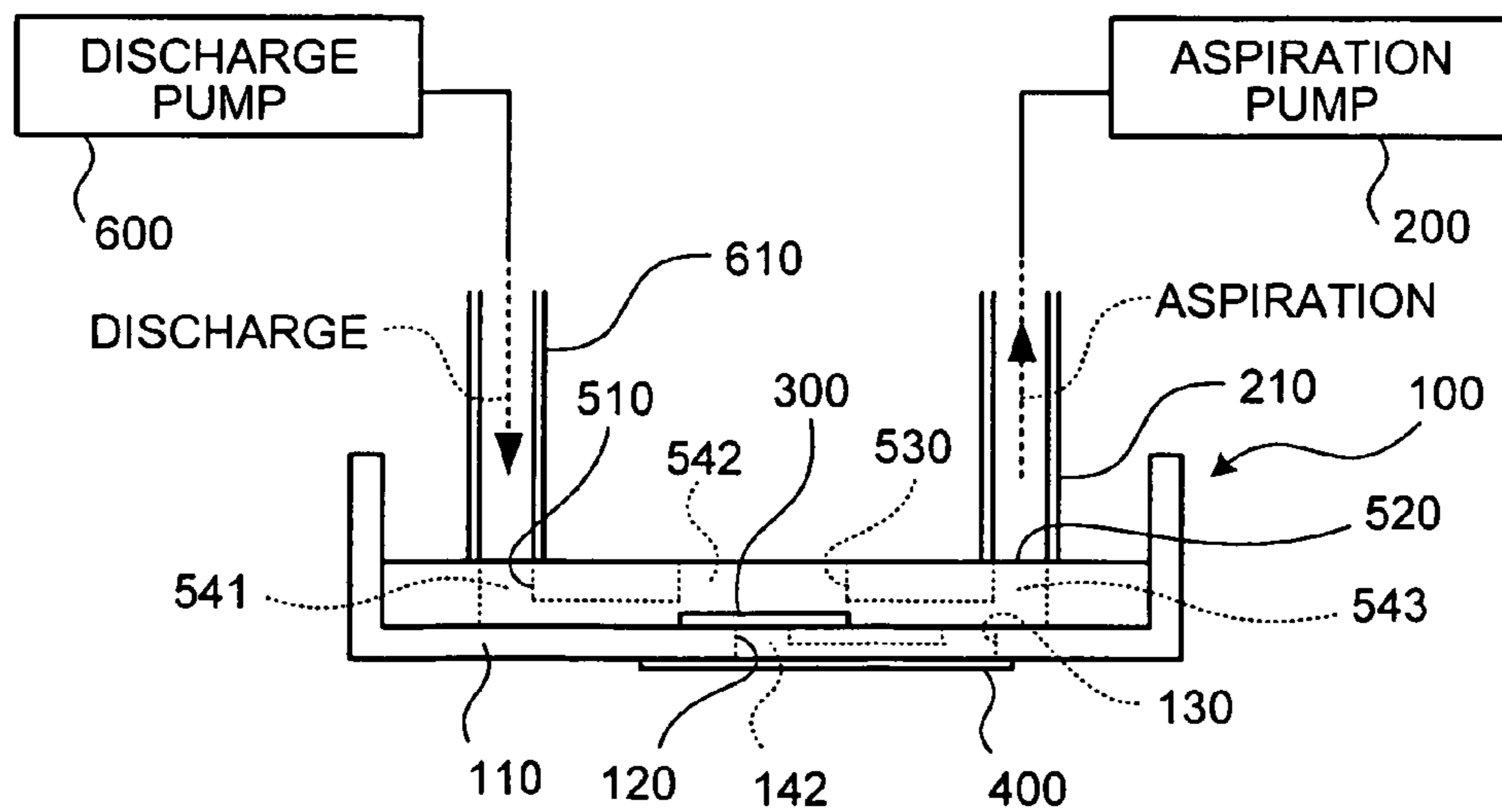


FIG.8A

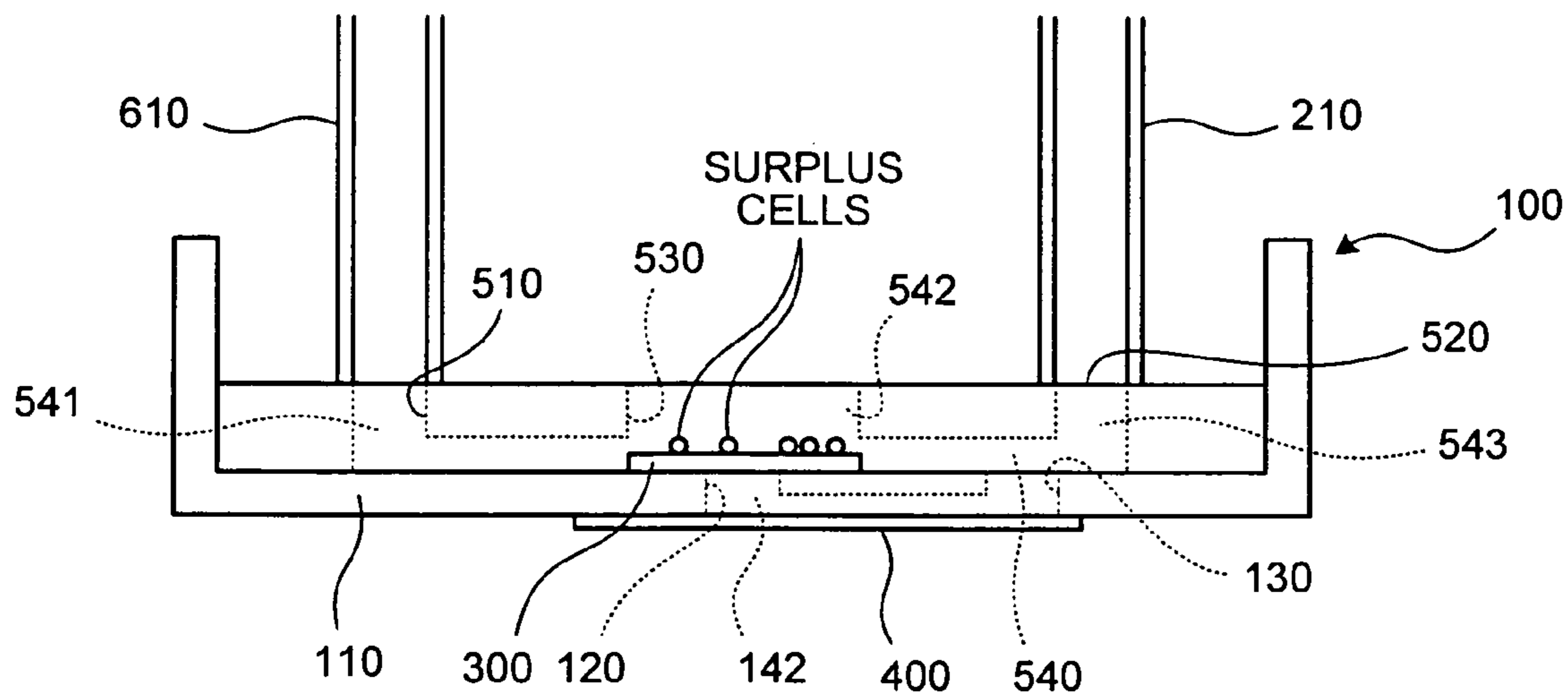


FIG.8B

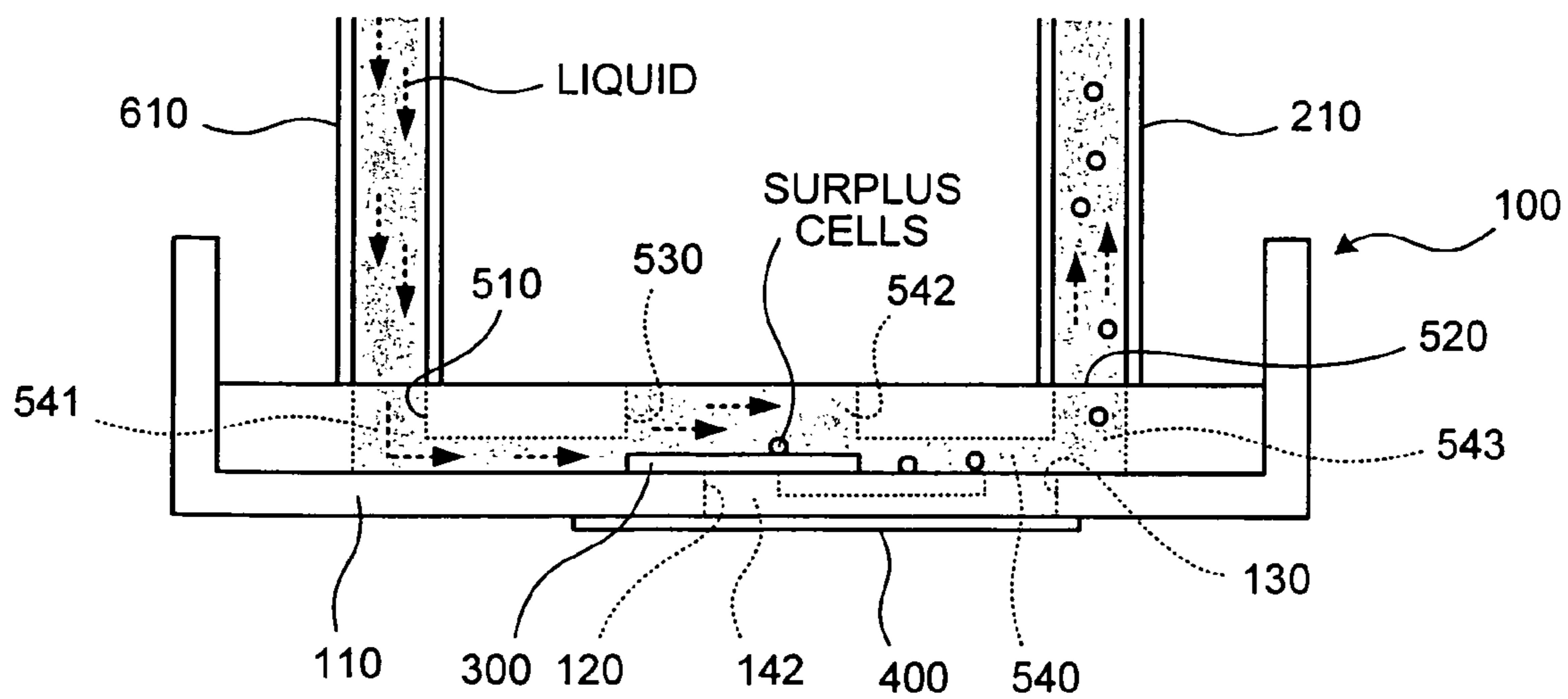


FIG.9A

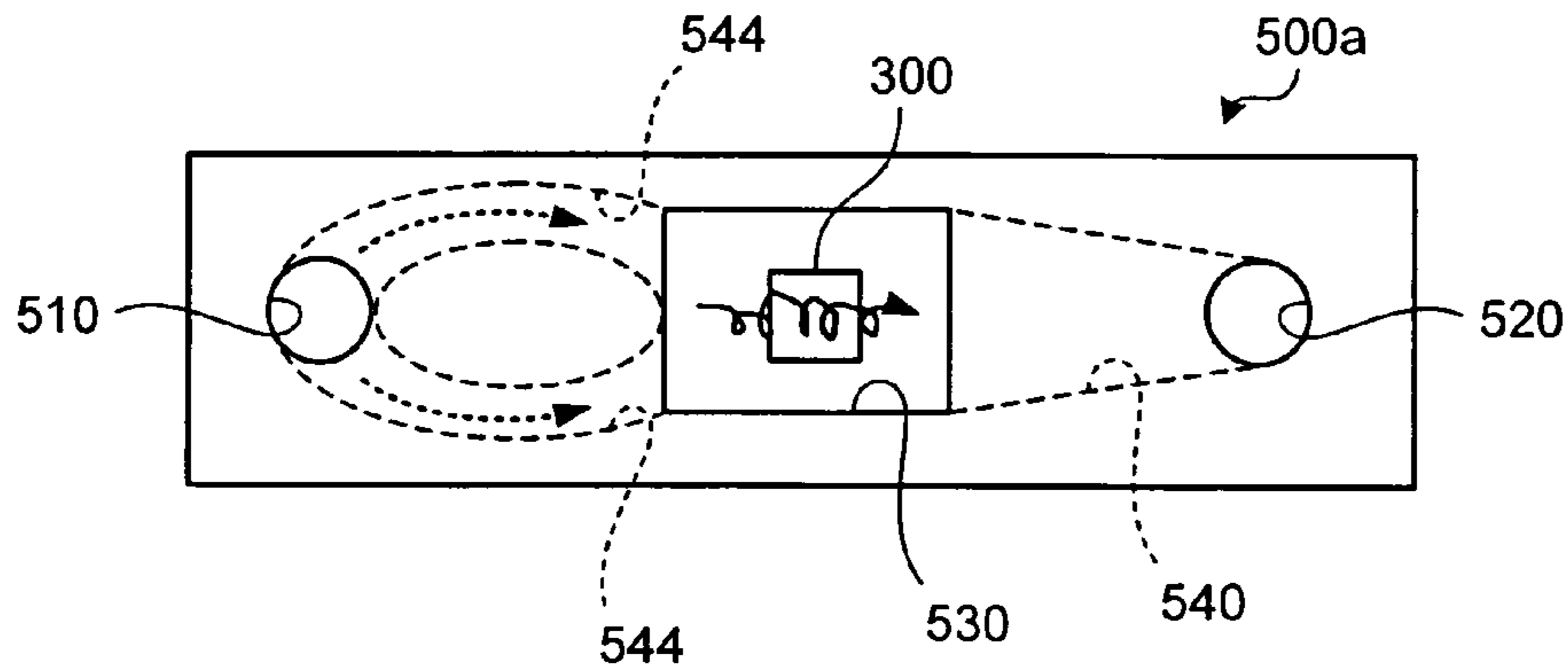


FIG.9B

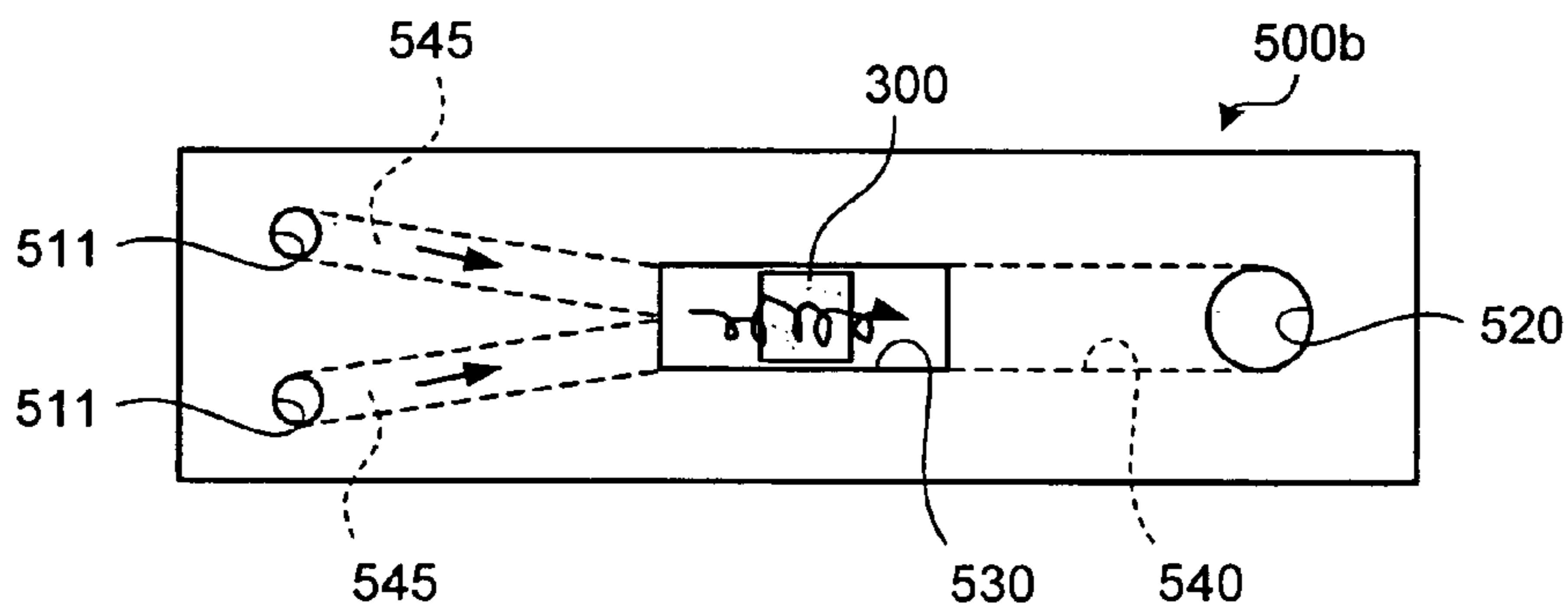


FIG.9C

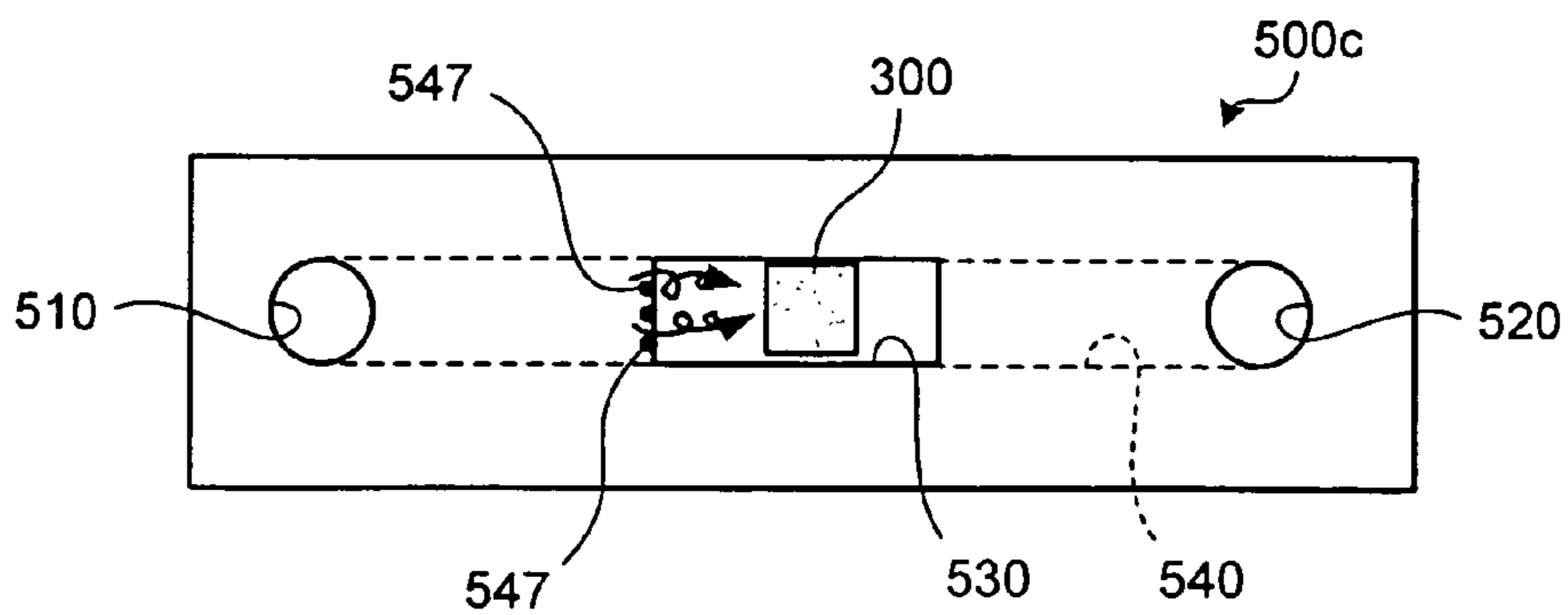


FIG. 10

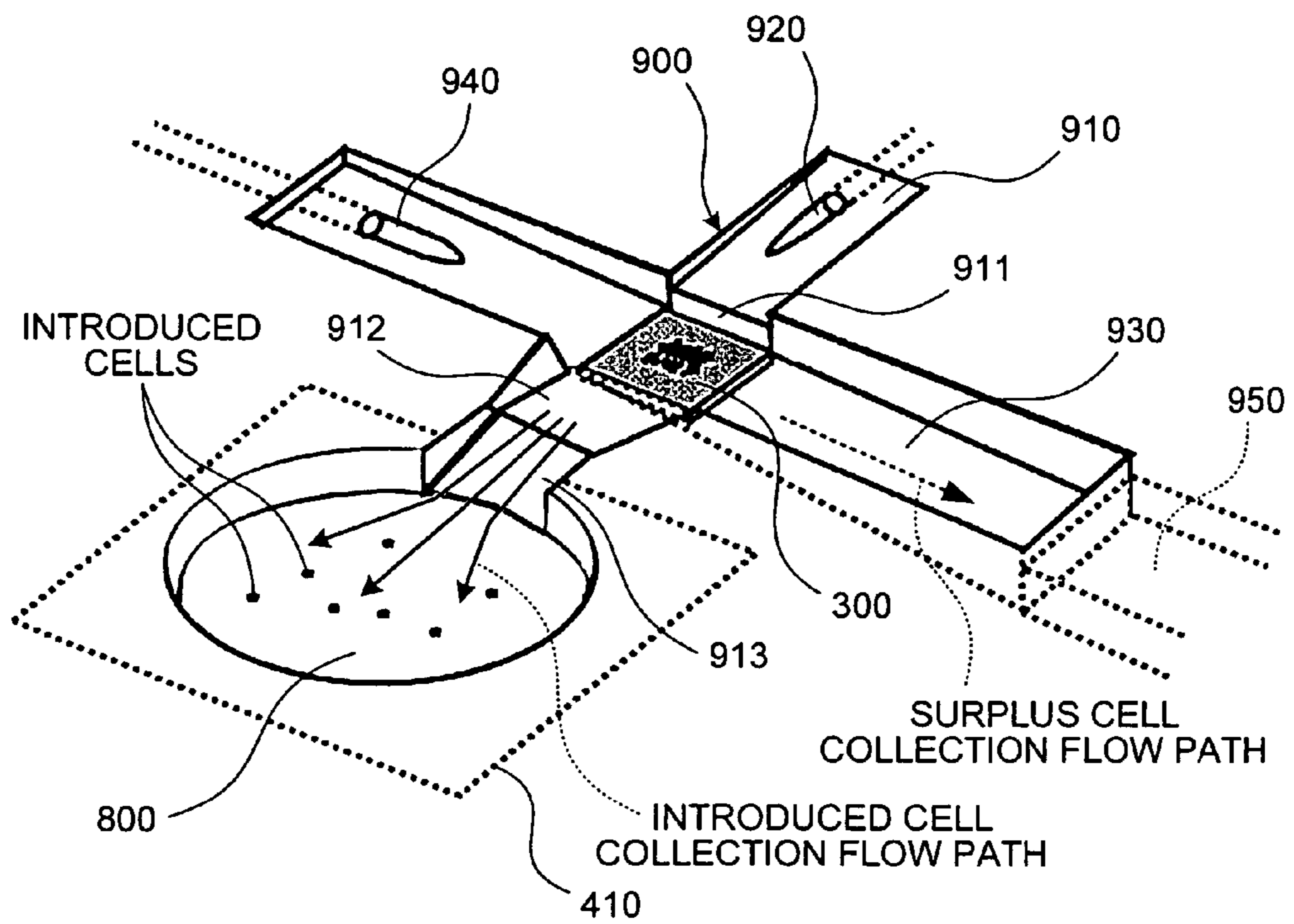


FIG. 11

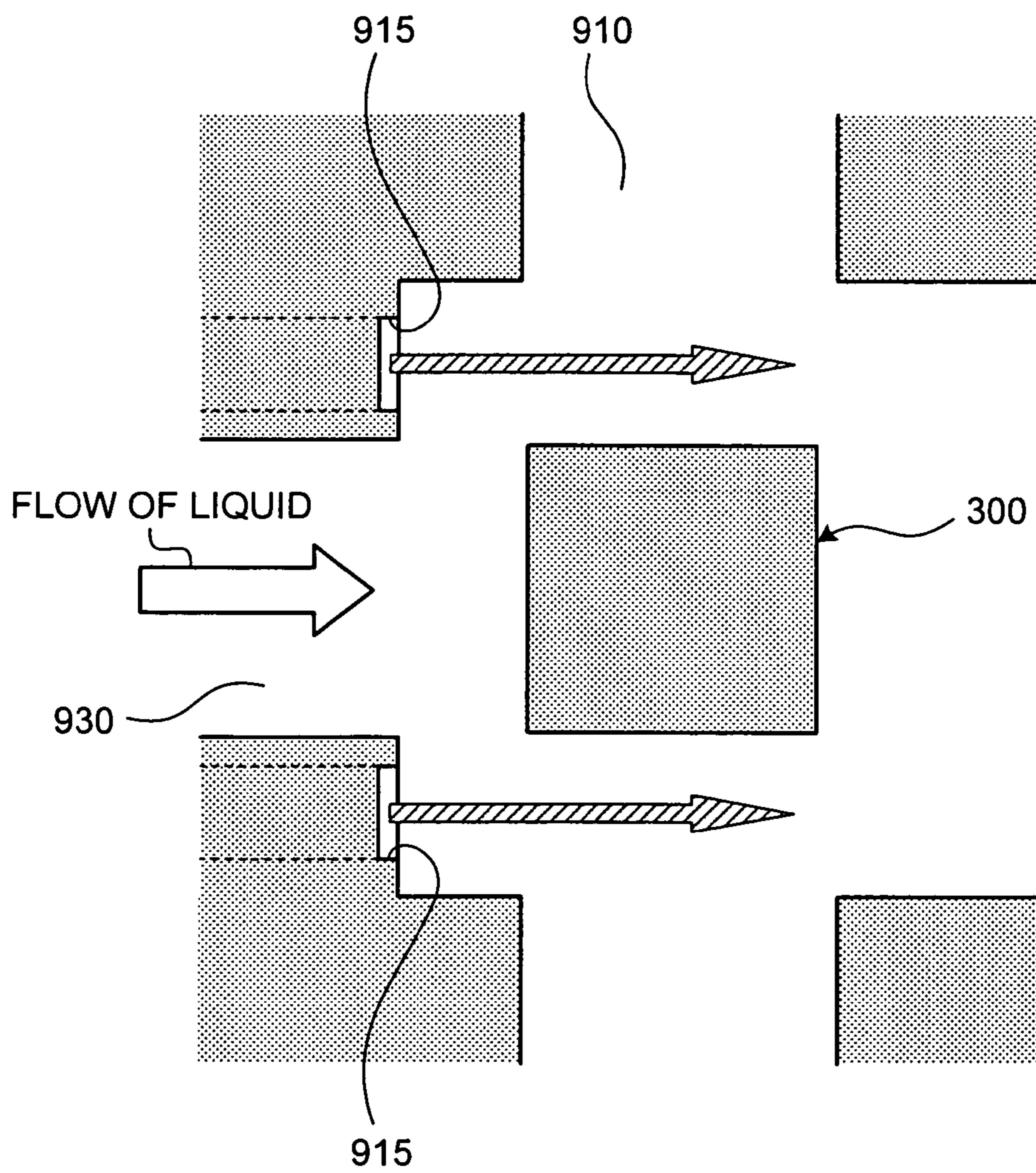


FIG.12

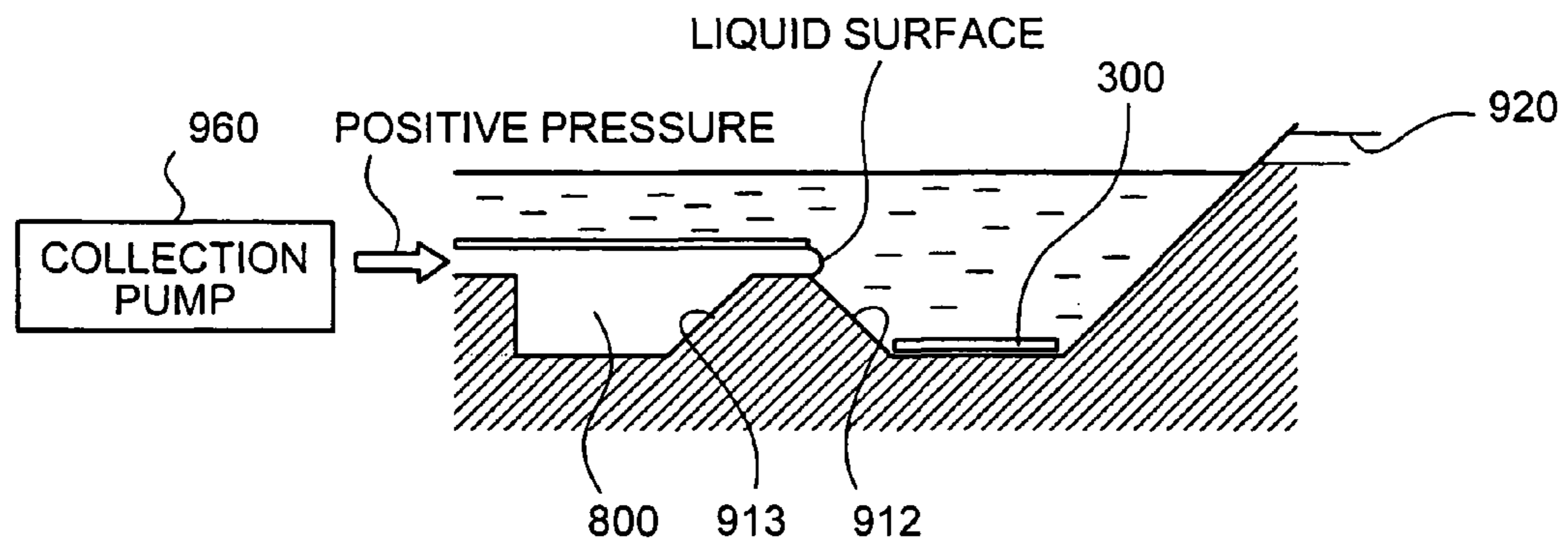


FIG.13

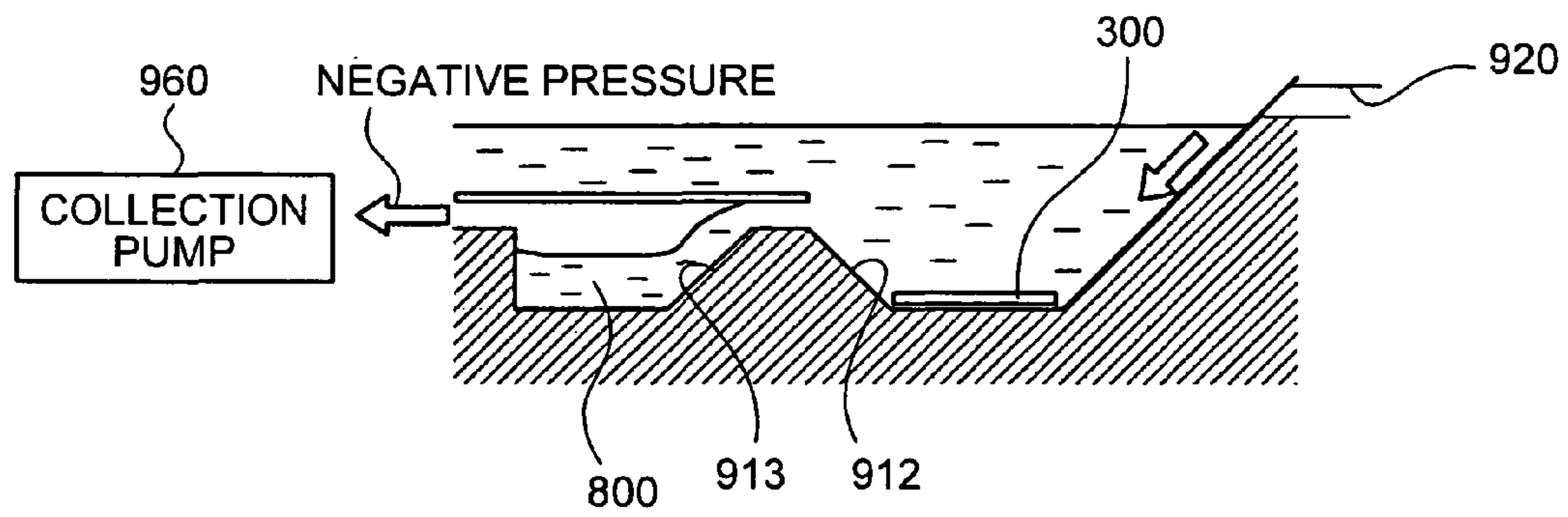


FIG. 14

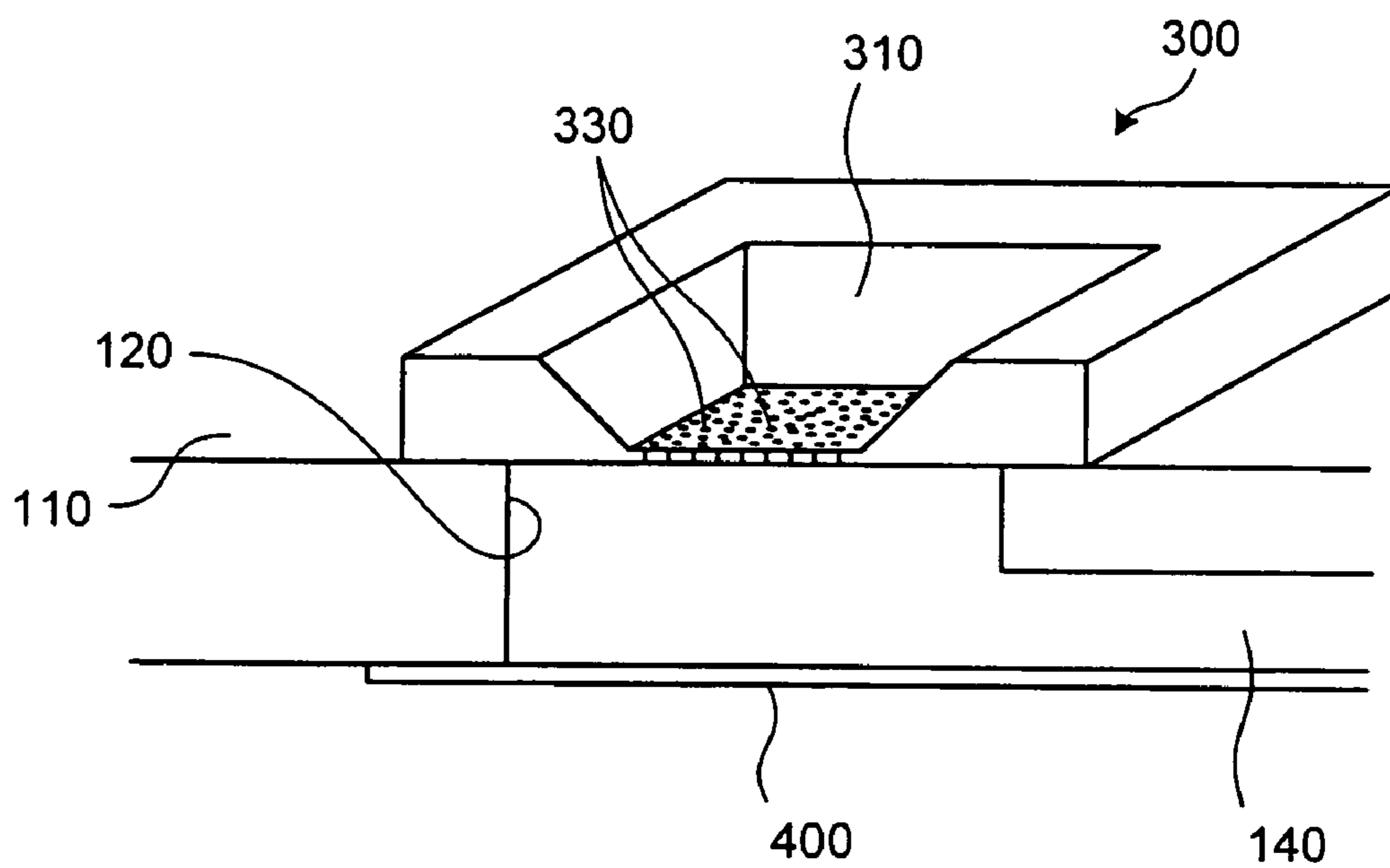


FIG. 15A

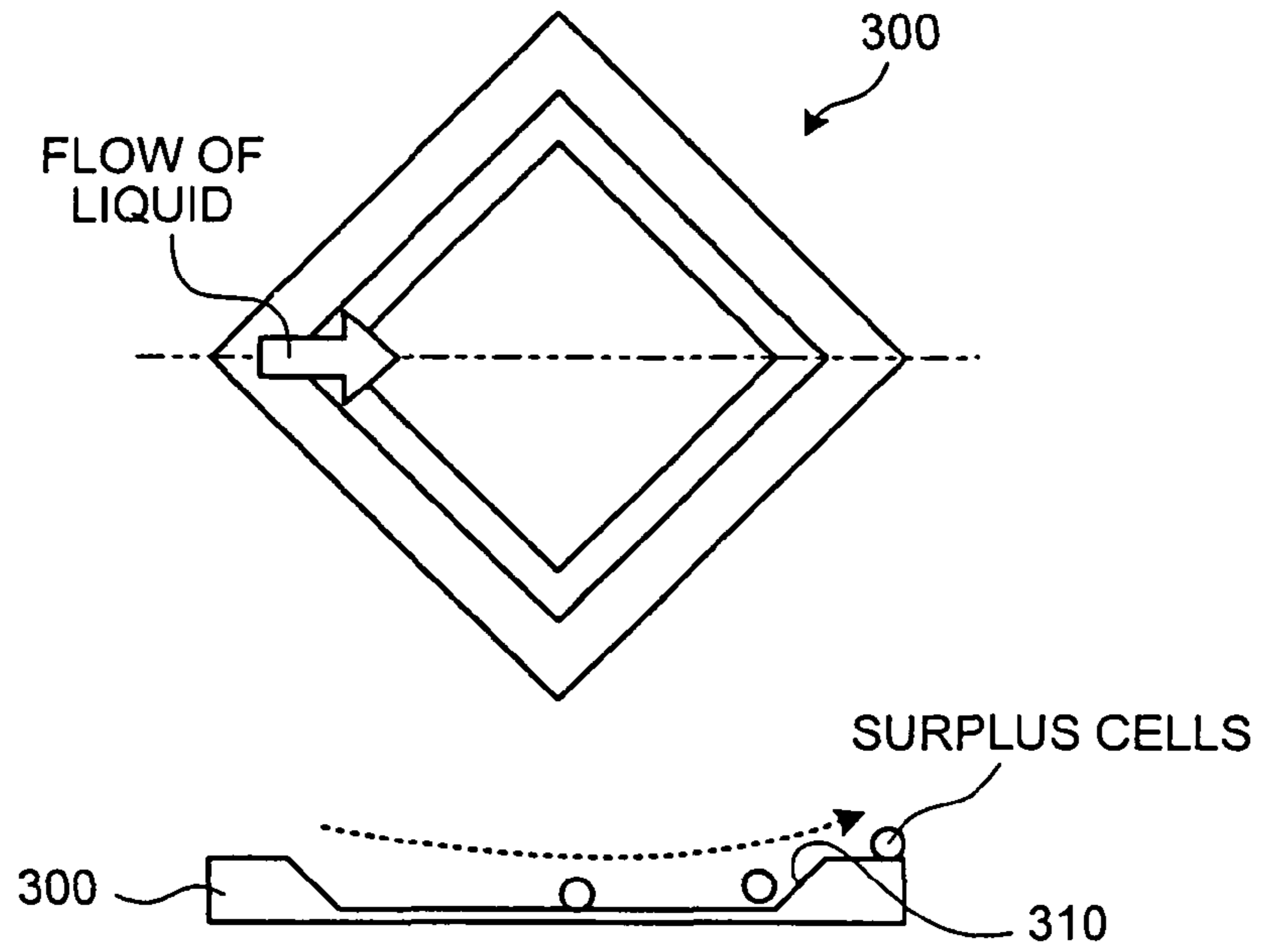


FIG. 15B

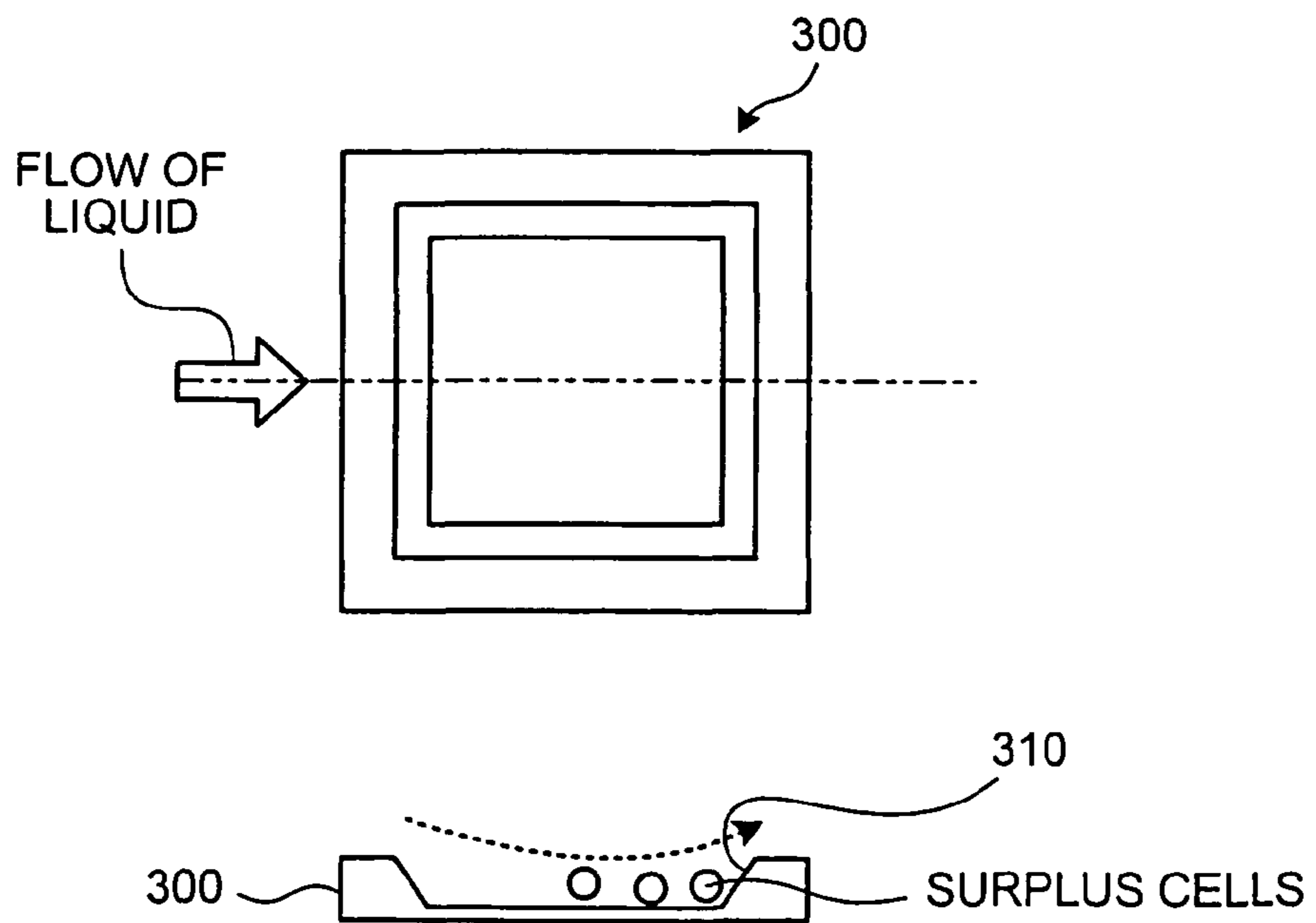


FIG.16

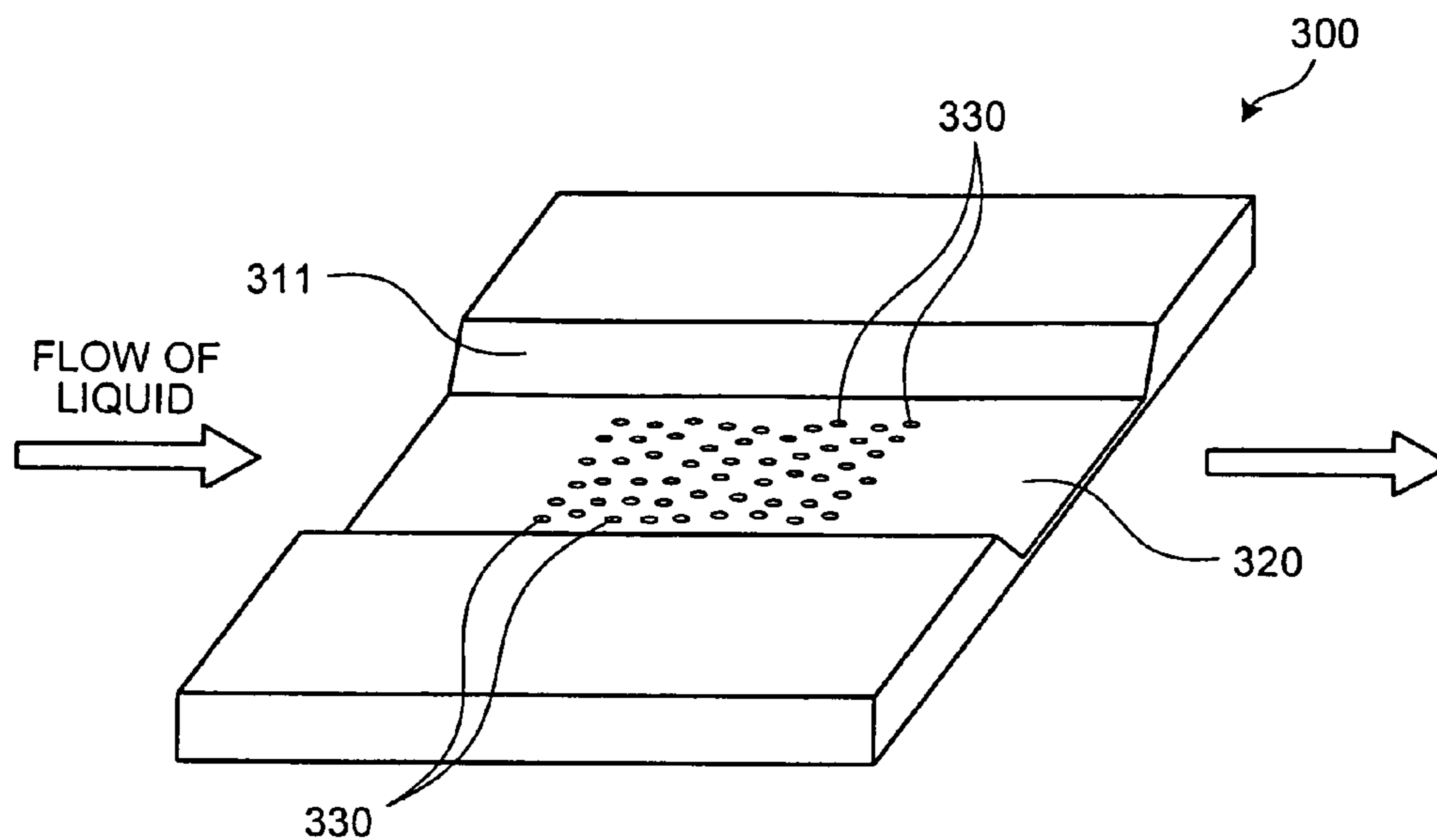
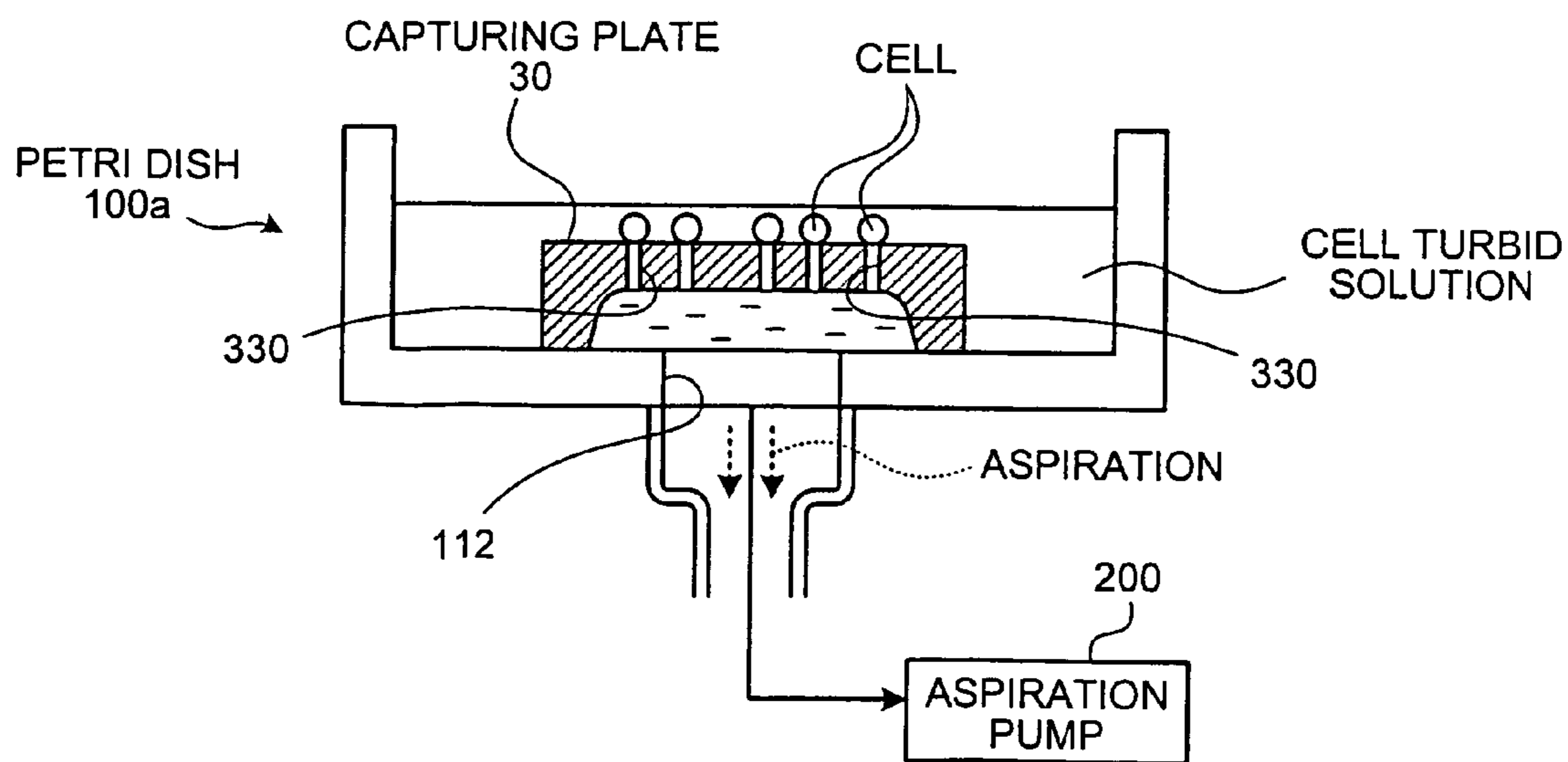


FIG.17



APPARATUS FOR CAPTURING CELL

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to an apparatus for capturing a cell for injecting a substance such as a gene or drug into the cell. More specifically, the invention relates to an apparatus that allows observation of the cell while the substance is being injected in the cell.

2. Description of the Related Art

The injection (introduction) of genes or drugs into cells using a needle to alter their properties has recently been carried out in the life science fields, and particularly in the fields of regenerative medicine and genome drug development. When injecting a gene or drug into a cell, it is necessary to immobilize the cell to prevent it from moving. A cell immobilizing plate (hereinafter, "cell capturing plate") has been conventionally used to immobilize a cell. The cell capturing plate has a plurality of microscopic capturing holes. The diameter of these holes is smaller than that of the cells. The diameter of the cells can vary from 10 micrometers to 100 micrometers depending on the type of the cells. Cells are caught, and thereby immobilized, in these holes when the cells, or a liquid containing the cells, is aspirated from the other side of the cell immobilizing plate with an aspiration pump.

A microinjection method has been developed as a method for injecting a gene or drug directly into a cell through a needle. In the microinjection method, an ultrafine needle having a diameter of 1 micrometer or less (tip outer diameter: 1 micrometer, inner diameter: about 0.5 micrometers) and filled with a drug is inserted into a cell and the drug is injected into the immobilized cell. This process is performed while observing the cell and the needle under a microscope.

Petri dishes used in the conventional cell capturing apparatus require a capturing plate that captures cells, a well for holding a cell turbid solution, and on the bottom, a connection for an aspiration tube from an aspiration pump used to aspirate cells into microscopic capturing holes in the capturing plate. Consequently, a structure is mainly employed for conventional Petri dishes for cells in which microscopic holes are formed at predetermined positions in a Petri dish, a cell capturing plate is adhered to form a well, and the Petri dish is placed on a unit that was machined to serve as an aspiration connector from an aspiration pump.

FIG. 17 is a schematic block diagram of a Petri dish used in a conventional cell capturing apparatus. A cell turbid solution is contained inside a Petri dish 100a, a through hole 112 is formed approximately in the center of the Petri dish 100a, and a capturing plate 30 is fixed on the through hole 112 by adhesion and the like.

A plurality of micropores 330 are formed in the capturing plate 30. Cells are captured in these micropores 330 by aspirating the cell turbid solution from below with an aspiration pump 200 through the through hole 112. The captured cells can be observed with a microscope (not shown) from above the Petri dish 100a.

Conventional techniques relating to capturing of cells have been disclosed, for example, in Japanese Patent Application Laid-open No. 2004-180555 and Japanese Patent Application Laid-open No. 2004-163.

In the conventional Petri dish 100a, there has been a problem of the occurrence of leakage from a gap between the Petri dish 100a and the capturing plate 30, thereby preventing the necessary cells from being aspirated (captured) reliably.

It is a common practice to observe the cells with a transmitted light. Since the well and aspiration connection are molded into a single unit as a result of machining in a conventional Petri dish as previously described, however, the machined surface on the bottom of the capturing plate cannot be optically polished, thereby resulting in the problem of being unable to observe the cells from below the capturing plate with transmitted light.

Furthermore, there are no particular considerations given to the handling of injected cells, which are cells in which a substance has been injected, and empty cells, which are cells in which the substance has not been injected because they are not suitable for injection of the substance. Since a technique capable of distinguishing between these injected cells and empty cells has not been examined, a method of distinguishing these injected cells and empty cells is desired.

SUMMARY OF THE INVENTION

It is an object of the present invention to at least solve the problems in the conventional technology.

According to an aspect of the present invention, a cell capturing apparatus, which is suitably used to capture a cell and inject a substance into captured cell while observing the cell under a microscope, includes a cell capturing container having a bottom wall and a side wall, the bottom wall including a first through hole; a second through hole; and a groove that connects ends of the first through hole and the second through hole that open on outer surface of the bottom wall; a transparent plate member that covers a portion of the outer surface of the bottom wall including at least the first through hole, the second through hole, and the groove; a capturing plate disposed inner surface of the bottom wall and above the first through hole; and an aspiration tube with one end connected to an upper portion of the second through hole and other end connected to an aspirating unit.

The above and other objects, features, advantages and technical and industrial significance of this invention will be better understood by reading the following detailed description of presently preferred embodiments of the invention, when considered in connection with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a top view of a Petri dish according to a first embodiment of the present invention;

FIG. 2 is a longitudinal cross-section of the Petri dish shown in FIG. 1;

FIG. 3 is a perspective view (front side view) of a capturing plate according to the first embodiment;

FIG. 4 is a perspective view (back side view) of the capturing plate shown in FIG. 3;

FIG. 5A is a top view of an empty-cell collection plate according to a second embodiment of the present invention;

FIG. 5B is a longitudinal cross-section of the empty-cell collection plate shown in FIG. 5A;

FIG. 6 is a top view of a Petri dish on which the empty-cell collection plate shown in FIG. 5A is mounted;

FIG. 7 is a longitudinal cross-section of a Petri dish on which the empty-cell collection plate shown in FIG. 5A is mounted along with some other relevant parts;

FIG. 8A is an explanatory diagram of the state prior to collection of empty cells;

FIG. 8B is an explanatory diagram of the state during collection of the empty cells;

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FIG. 9A is a top view of an empty-cell collection plate according to a first example;

FIG. 9B is a top view of an empty-cell collection plate according to a second example;

FIG. 9C is a top view of an empty-cell collection plate according to a third example;

FIG. 10 is a perspective view of a multifunctional flow path plate according to a third embodiment of the present invention with other relevant parts;

FIG. 11 is a top view of relevant parts of the multifunctional flow path plate shown in FIG. 10;

FIG. 12 is a longitudinal cross-section of the state prior to collection of injected cells in the multifunctional flow path plate shown in FIG. 10;

FIG. 13 is a longitudinal cross-section of the state during collection of the injected cells in the multifunctional flow path plate shown in FIG. 10;

FIG. 14 is a perspective view of a capturing plate according to a fourth embodiment of the present invention;

FIG. 15A is an explanatory diagram to explain an example of an orientation of a capturing plate according to a fifth embodiment of the present invention;

FIG. 15B is an explanatory diagram to explain another example of an orientation of the capturing plate;

FIG. 16 is a perspective view of a capturing plate according to a sixth embodiment of the present invention; and

FIG. 17 is a longitudinal cross-section of relevant parts of a conventional Petri dish for capturing cells.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Exemplary embodiments of the present invention are explained below in detail with reference to the accompanying drawings. The following explains a summary and characteristics of the structure of the Petri dish according to each embodiment. It is followed by a detailed explanation of the functions resulting from the constitution of the Petri dish, and the constitutions and functions relating to an empty-cell collecting member mounted to the Petri dish and a multifunctional flow path plate.

A Petri dish 100 according to a first embodiment is explained below. This Petri dish 100 is characterized in preventing leakage, which has been a problem in the conventional techniques, and facilitating observation of captured cells.

An aspiration system is provided above the Petri dish 100, and together with aspirating cells from above, a flow path formed on the Petri dish 100 is made to facilitate observation of captured cells by sealing with a transparent plate member 400 from below the Petri dish 100.

The Petri dish 100 is explained in detail with reference to FIGS. 1 and 2. FIG. 1 is a top view and FIG. 2 is a longitudinal cross-section of the Petri dish 100.

Namely, as shown in FIGS. 1 and 2, the Petri dish 100 is formed from a circular base (a bottom wall 110) and a peripheral wall 111, and two through holes 120 and 130, which are mutually separated and open upward, and an intake flow path 140 for aspirating cells, are formed on the bottom wall 110 of the Petri dish 100. The intake flow path 140 is composed by connecting a first flow path 141 continuous with the through hole 120, a second flow path 142, and a third flow path 143 continuous with the through hole 130.

The lower side of the intake flow path 140 is sealed by the transparent plate member 400. Accordingly, a closed flow path is formed within the intake flow path 140 through which intake air passes from the aspiration pump 200. Furthermore,

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although the intake direction of the intake flow path 140 is provided on the upper side in this example, the intake flow path 140 can be also made to, for example, intake from the horizontal direction of the Petri dish 100.

A capturing plate 300 is fixed by adhesion and the like to the portion corresponding to the formed position of the through hole 120 approximately in the center of the bottom wall 110. More specifically, the attached position of the capturing plate 300 is selected so that the through hole 120 is positioned approximately in the center of the capturing plate 300.

This is because cells on the capturing plate 300 are reliably aspirated through a plurality of micropores 330 formed on the capturing plate 300 as will be described later. On the other hand, an aspiration tube 210 for intake of air from the intake flow path 140 is attached by adhesion and the like to upwardly opening the through hole 130 through a coupling (not shown).

The transparent plate member 400 is sealed by affixation to the position below the capturing plate 300 where the through hole 120 and the second flow path 141 are formed. Consequently, the transparent plate member 400 has a function of serving as a distribution route by covering the intake flow path 140 formed on the Petri dish 100 from below, and a function for observing cells with transmitted light.

The characteristics and structure of the capturing plate 300 shown in FIGS. 1 and 2 are explained in detail with reference to FIGS. 3 and 4. FIG. 3 is a perspective view (front side view) of the capturing plate 300 attached to the Petri dish 100. FIG. 4 is a perspective view (back side view) of the capturing plate 300 in an inverted state as viewed from the side of the transparent plate member 400.

Namely, as shown in FIGS. 3 and 4, the capturing plate 300 is composed in the shape of a square, an upwardly opening rectangular concave portion 310 is formed approximately in its center, and a plurality of the micropores 330 are randomly formed on the lower surface 320 of the concave portion 310. The micropores 330 are formed for the purpose of capturing cells through the first flow path 141 of the intake flow path 140 with intake air that has been aspirated through the aspiration pump 200 and the aspiration tube 210. This aspiration allows cells to be captured in the capturing plate 300.

The diameter of the micropores 330 is determined according to the size of the cells to be captured. For example, in the case of attempting to capture cells having a diameter of about 10 to 20 micrometers, the diameter of the micropores 330 is set to that which is smaller than the diameter of these cells.

Since the micropores 330 formed on the capturing plate 300 have a large aspect ratio, the portion where the micropores 330 are formed must be thin. However, since reducing the thickness of the entire capturing plate 300 results in decreased strength, in this example, together with using a silicon material for the composite material of the capturing plate 300, only the portion where the micropores 330 are formed (the lower surface 320) is made to be thin. The use of such a structure makes it possible to maintain the strength of the capturing plate 300 itself.

As has been explained above, according to the first embodiment, the Petri dish 100 employs a constitution that a pair of the through holes 120 and 130 are formed at two positions on the bottom wall 110, the intake flow path 140 is formed that connects the lower openings of these through holes 120 and 130, the transparent plate member 400 is provided that seals the intake flow path 140 from below the bottom wall 110, the capturing plate 300 is disposed at the position of the upper opening formed by the through hole 120, and the aspiration tube 210 continuous with the aspiration pump 200 is connected to the upper opening of the through hole 130. Thus,

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cells can be reliably aspirated without the occurrence of problems such as leakage. Cells can be also easily observed with transmitted light through the transparent plate member **400**.

A second embodiment of a cell capturing apparatus of the present invention is explained in detail. Namely, as shown in FIGS. **5A**, **5B**, **6**, and **7**, the second embodiment is characterized by having an empty-cell collection plate **500** for collecting empty cells mounted on the Petri dish **100** in addition to an aspiration route (the intake flow path **140**) formed at the bottom of the Petri dish **100**.

Injected cells and empty cells are both present on the surface of the capturing plate **300**. Thus, in the second embodiment, by mounting the empty-cell collection plate **500** within the Petri dish **100**, these empty cells can be collected and removed efficiently.

The characteristics and functions of the empty-cell collection plate **500** are explained in detail with reference to FIGS. **5A**, **5B**, **6**, and **7**. FIG. **5A** is a top view and FIG. **5B** is a longitudinal cross-section of the constitution of the empty-cell collection plate **500**. FIG. **6** is a top view of the Petri dish **100** on which the empty-cell collection plate **500** is mounted, and FIG. **7** is their respective longitudinal cross-sections. As shown in FIGS. **6** and **7**, the empty-cell collection plate **500** is attached by adhesive and the like to the bottom wall **110** of the Petri dish **100**.

As shown in FIGS. **5A**, **5B**, **6**, and **7**, the empty-cell collection plate **500** is composed in the shape of a square having nearly the same dimensions as the inner diameter of the Petri dish **100**, and a pair of the through holes **510** and **520** at both ends and an injection opening **530** approximately in the center are formed together with a collection flow path **540**.

Two through holes **510** and **520**, which are mutually separated and open upward, and the collection flow path **540**, through which collection liquid flows, are formed on the empty-cell collection plate **500**, and the collection flow path **540** is composed by connecting a first flow path **541** continuous with the through hole **510**, a second flow path **542**, and a third flow path **543** continuous with the through hole **520**.

Since the bottom of the collection flow path **540** is sealed by the upper surface of the bottom wall **110** of the Petri dish **100**, a flow path is formed that distributes collection liquid within the empty-cell collection plate **500**.

The through hole **510** functions as a liquid (cell turbid solution, culture liquid, or physiological saline) discharge hole, and a discharge tube **610** of a discharge pump **600** for discharging cleaning water is connected to the through hole **510**. The aspiration tube **210** of the aspiration pump **200** is connected to the through hole **520**.

Collection of the empty cells by the empty-cell collection plate **500** is explained with reference to FIGS. **8A** and **8B**. FIG. **8A** is a diagram of the state prior to collecting the empty cells, and at this time, as shown in FIG. **8B**, as a result of physiological saline supplied from the discharge pump **600** flowing through the discharge tube **610** and passing through the first flow path **541** continuous with the through hole **510**, the second flow path **542**, and the third flow path **543** continuous with the through hole **520**, the empty cells near the capturing plate **300** flow and are collected at the outside through the aspiration tube **210**.

As a result, unnecessary empty cells can be collected efficiently while allowing the necessary injected cells to remain in the cell capturing plate **300**. The liquid discharged as collection liquid from the discharge pump **600** (cell turbid solution, culture liquid, or physiological saline) can use the same liquid as the liquid used for injection.

Although the function of the empty-cell collection plate **500** of the second embodiment is mainly to collect the empty

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cells, it can be also used as an injection liquid supply apparatus for supplying a liquid (cell turbid solution, culture liquid, or physiological saline) to the Petri dish **100** when simply carrying out cell injection.

As has been explained above, since the second embodiment is composed by mounting the empty-cell collection plate **500** for collecting unnecessary empty cells within the Petri dish **100**, in addition to being able to easily distinguish between the injected cells and the empty cells within the Petri dish **100**, which has not been achieved by the conventional techniques, the necessary injected cells remain in the capturing plate **300**, while unnecessary empty cells can be efficiently collected.

Variations of the empty-cell collection plate of the second embodiment are explained in detail with reference to FIGS. **9A** to **9C**. FIG. **9A** is a first example of the structure of an empty-cell collection plate **500a**. As shown in FIG. **9A**, this example is characterized by employing a constitution that the flow path from the through hole **510** to the capturing plate **300** is in the form of a branched flow paths **544**, and by supplying liquid from these flow paths **544** (multiple discharge flow paths), an eddy flow path is formed near the position where the capturing plate **300** is disposed.

Since liquid flows in from these multiple flow paths **544**, eddy flow is generated near the capturing plate **300**, thereby enabling empty cells to be removed. Accordingly, only the necessary cells can be captured.

FIG. **9B** is a second example of the structure of the empty-cell collection plate **500b**. As shown in FIG. **9B**, this example employs a constitution that flow paths **545**, **545** respectively connected to two through holes **511**, **511** form a branched flow path. In this second example, since the force of the liquid that flows from the flow paths **545**, **545** in two directions increases, empty cells can be removed more effectively.

In this case, when discharging liquid from each of the two through holes **511**, **511**, the characteristics of each liquid can be changed. For example, eddy flow can be generated more easily by changing conditions such as concentration and temperature.

FIG. **9C** is a third example of the structure of the empty-cell collection plate **500c**. As shown in FIG. **9C**, this example is characterized by providing a plurality of convex portions **547** at the position of the initial flow path that leads to the capturing plate **300**. Namely, since these convex portions **547** serve as obstructions in the flow path, when water that flows in from the first flow path **541** has reached these convex portions **547**, the flow path is dispersed, resulting in the formation of eddy flow near the capturing plate **300** and enabling empty cells to be removed more effectively.

A third embodiment of the cell capturing apparatus according to the present invention is explained in detail with reference to FIG. **10**. FIG. **10** is a perspective view of the constitution of a multifunctional flow path plate **900**. As shown in FIG. **10**, the third embodiment is characterized by employing a constitution that the multifunctional flow path plate **900** is provided in the cell capturing apparatus, and the multifunctional flow path plate **900** includes (1) a function that supplies cells, culture liquid and the like, (2) a function that collects empty cells, (3) a function for allowing cells that have been injected (injected cells) to flow, and (4) a function that collects the injected cells.

Namely, as shown in FIG. **10**, the multifunctional flow path plate **900** has an injected-cell collecting flow-path **910** for allowing injected cells to flow and collecting those injected cells, and an empty-cell collecting flow-path **930** for allowing empty cells to flow and collecting those empty cells, that cross nearly perpendicularly. The capturing plate **300** is provided at

the position (center) where the injected-cell collecting flow-path **910** and the empty-cell collecting flow-path **930** respectively cross.

A discharge port **920** from which liquid is discharged is formed in the upstream end (upper side of FIG. **10**) of the injected-cell collecting flow-path **910**, and an observation well **800** formed into a circular shape for observing cells is provided at the downstream end (lower side of FIG. **10**). Introduced cells that have flowed from the capturing plate **300** are made to flow into the observation well **800** by liquid discharged from the discharge port **920**, and a transparent plate member **410** that uses a glass material and the like is affixed to the bottom of the observation well **800**. As a result, injected cells that have flowed into the observation well **800** can be easily observed with transmitted light or fluorescent light through the transparent plate member **410**.

A first inclination **912**, which inclines upward from the position of the upper surface of the capturing plate **300**, and a second inclination **913**, which inclines downward from the apex of the first inclination **912** towards the observation well **800**, are provided between the capturing plate **300** and the observation well **800**. In other words, by providing the first and the second inclinations **912** and **913** at an intermediate position in the flow path from the capturing plate **300** to the observation well **800**, injected cells that have flowed into the observation well **800** are effectively prevented from flowing back outside the observation well **800**.

Furthermore, a step **911**, the position of which lowers as it approaches the capturing plate **300**, is provided in the multifunctional flow path plate **900** at the position where the flow path for collecting injected cells and the flow path for collecting empty cells intersect to prevent mixing of empty cells and injected cells.

A discharge port **940** from which liquid is discharged is formed in the upstream end (left side of FIG. **10**) of the empty-cell collecting flow-path **930**, and an empty cell collection port **950** for collecting empty cells is provided in the downstream end (right side of FIG. **10**). Surplus cells present near the capturing plate **300** are made to flow into the empty cell collection port **950** by liquid discharged from the discharge port **940**.

During collection of injected cells and empty cells by the multifunctional flow path plate **900**, the times at which liquid is allowed to flow differ between the case of collecting injected cells and the case of collecting empty cells. In the case of supplying cells or culture liquid to the Petri dish **100**, either the liquid discharge port **920** or the liquid discharge port **940** is used.

FIG. **11** is a top view of a shield flow path for preventing mixing of the empty cells and the injected cells. Namely, as shown in FIG. **11**, the third embodiment is characterized by respectively forming discharge ports **915** from which cleaning liquid flows at both ends of the discharge port **920** for removal of the empty cells. More specifically, liquid is first made to flow from a pair of the liquid discharge ports **915** provided on the left and right sides before carrying out an empty cell removal step.

As a result, since a so-called shield flow path (a curtain of path) is formed, the flow of the empty cells into the discharge port **920** for injected cells and the discharge port **940** for collection in the observation well **800** can be reliably prevented. At this time, since the flow rate when injected cells are allowed to flow is faster than the flow rate when the empty cells are allowed to flow, shielding effects are further improved, thereby making it possible to prevent mixing of the empty cells and the injected cells.

An example of a collection method for injected cells using the multifunctional flow path plate **900** is explained with reference to FIGS. **12** and **13**. FIG. **12** is a cross-section of relevant parts shown in FIG. **11**, and depicts the state prior to collecting injected cells. FIG. **13** is also a cross-section of the relevant parts shown in FIG. **11**, and depicts the state during collection of injected cells. Namely, in this example, a collection pump **960** for collecting injected cells is provided at one end of the well **800**, and by varying the aspiration pressure generated by the collection pump **960** (between positive pressure and negative pressure), the injected cells and the empty cells are prevented from mixing. More specifically, the collection pump **960** is controlled so as to generate positive aspiration pressure prior to collecting injected cells as shown in FIG. **12**.

In this case, as shown in FIG. **12**, liquid does not penetrate into the observation position of the well **800**. Accordingly, liquid can be prevented from flowing into the well **800**. On the other hand, the collection pump **960** is controlled by switching its aspiration pressure from positive pressure to negative pressure when collecting injected cells. Accordingly, as shown in FIG. **13**, injected cells flow into the well **800** together with a cell turbid solution discharged from the liquid discharge port **920** (FIG. **11**), enabling these injected cells to be observed.

A fourth embodiment of the present invention is explained in detail with reference to FIG. **14**. The fourth embodiment is characterized by employing a method of fixing the capturing plate **300** to the Petri dish **100** (joined orientation of the capturing plate **300**). Namely, when observing cells by transmitted light with a microscope provided in the cell capturing apparatus, although a cell turbid solution (culture liquid) present in the concave portion **310** of capturing plate **300**, air and the transparent plate member **400** are present between the capturing plate **300** and the objective lens of the microscope, if air bubbles are present between the transparent plate member **400** and the capturing plate **300**, cells cannot be observed satisfactorily. Thus, cells are observed in the state in which culture liquid is filled between the transparent plate member **400** and the capturing plate **300**.

Since the optical resolution during microscopic observation is affected by the thickness of the culture liquid, when culture liquid is filled into the concave portion **310** of the capturing plate **300**, optical resolution decreases. Decreases in optical resolution can therefore be prevented by reducing the thickness (amount filled) of the culture liquid. In focusing on this point, the fourth embodiment employs a constitution that the separation distance between the transparent plate member **400** adhered to the bottom of the Petri dish **100** and the capturing plate **300** is shortened.

More specifically, as shown in FIG. **14**, differing from the orientation in which the capturing plate **300** is joined as shown in the first embodiment, the lower surface **320** of the capturing plate **300** contacts the bottom wall **110** of the Petri dish **100**. According to the constitution of the fourth embodiment, the distance between the transparent plate member **400** and the capturing plate **300** can be shortened, and the thickness to which culture liquid is filled can therefore be reduced. Accordingly, decreases in optical resolution of the culture liquid during observation can be prevented.

A constitution can be also employed in which the lower surface of the capturing plate **300** is made to be slightly embedded in the bottom wall **110** of the Petri dish **100**, thereby making it possible to shorten the distance between the transparent plate member **400** and the capturing plate **300**.

As has been explained above, since the fourth embodiment is composed by allowing the lower surface of the capturing

plate **300** to contact the bottom wall **110** of the Petri dish **100**, the separation distance between the transparent plate member **400** and the capturing plate **300** can be shortened, and the thickness to which culture liquid is filled can therefore be reduced, thereby making it possible to prevent decreases in optical resolution during microscopic observation.

A fifth embodiment of the present invention is explained in detail with reference to FIGS. **15A** and **15B**. FIG. **15A** is a schematic view of the direction in which the capturing plate **300** is joined to the Petri dish **100** according to the fifth embodiment, and FIG. **15B** is a schematic view of the direction in which the capturing plate **300** is joined to the conventional Petri dish **100**.

As shown in FIG. **15B**, although the capturing plate **300** is provided such that it is disposed nearly in parallel with the bottom wall **110** of the Petri dish **100**, in this case, there is a certain degree of impairment of the empty cells collected by crossing the concave portion **310** in escaping due to the walls of the concave portion **310** during collection of the empty cells.

In focusing on this point, as shown in FIG. **15A**, the fifth embodiment sets the direction of placement of the capturing plate **300** in the Petri dish **100** so as to be positioned where it is oriented at an angle of about 45 degrees to the flow of liquid (rotated on an angle to the liquid flow path). Namely, as shown in FIG. **15A**, by setting the orientation of the capturing plate **300** to be about at an angle of 45 degrees, the angle of the walls of the concave portion **310** become smaller, thereby facilitating removal of the empty cells.

As has been explained above, since the direction in which the capturing plate **300** is placed in the Petri dish **100** in the fifth embodiment is set so as to be positioned at an angle of about 45 degrees, the flow during removal (collection) of the empty cells and collection of injected cells become linear, and in addition to enabling the collection efficiency of the empty cells to be improved, defective collection of the empty cells can be prevented.

A sixth embodiment of the present invention is explained in detail with reference to FIG. **16**. FIG. **16** is a schematic diagram of the constitution of the capturing plate **300** according to the sixth embodiment. Namely, as shown in FIG. **16**, by making one of the thick portions of the concave portion formed on the capturing plate **300** to be thin, a part of the concave portion serves characteristically as a concave portion **311** that distributes the flow of liquid.

In the case of the capturing plate **300** according to the sixth embodiment, by forming a flow path in the capturing plate **300** by reducing the thickness of a thick portion of the capturing plate **300**, in addition to resulting in a linear flow during removal (collection) of the empty cells and collection of injected cells, the collection efficiency of the empty cell and injected cells can be improved.

As has been explained above, since a distribution route is formed in the direction of flow in which liquid is distributed in one of the concave portions formed on the capturing plate **300** in the sixth embodiment, the flow of the removal (collection) of the empty cells and collection of injected cells becomes linear, thereby making it possible to improve the collection efficiency of the empty cells and injected cells.

According to an aspect of the present invention, it is possible to reliably prevent leakage. Moreover, it is possible to reliably and rapidly inject a predetermined amount of a substance into a large number of single cells. Furthermore, it is possible to observe cells easily and reliably with a transmitted light.

Moreover, because a fabrication step is carried out in which the back of the bottom wall of the cell capturing container is

machined, and the formed flow groove is sealed with a transparent plate member, an aspiration flow path for capturing cells can be fabricated easily without causing increased costs.

Furthermore, because the aspiration flow path formed inside the cell capturing container employs a constitution that aspirates liquid with an aspirating unit disposed above, a microscope can be disposed below the transparent plate member. Accordingly, problems such as a contact between the needle used to aspirate cells and the microscope can be eliminated.

Moreover, injected cells and empty cells can be distinguished easily and reliably.

Furthermore, in addition to injected cells and empty cells inside the Petri dish being able to be distinguished easily, which has been unable to be carried out in conventional techniques, the necessary injected cells remain in the cell capturing plate, while unnecessary empty cells can be collected efficiently.

Moreover, mixing of empty cells and injected cells can be reliably prevented by improving the shielding action between the injected-cell collecting flow-path and the empty-cell collecting flow-path.

Furthermore, the separation distance between the transparent plate member and capturing plate can be reduced, the thickness at which the culture liquid (cell turbid solution) is filled can be reduced. Accordingly, decreases in optical resolution during microscopic observation can be prevented.

Although the invention has been described with respect to a specific embodiment for a complete and clear disclosure, the appended claims are not to be thus limited but are to be construed as embodying all modifications and alternative constructions that may occur to one skilled in the art that fairly fall within the basic teaching herein set forth.

What is claimed is:

1. A cell capturing apparatus suitably used to capture a cell and inject a substance into captured cell while observing the cell under a microscope, comprising:

a cell capturing container having a bottom wall and a side wall, the bottom wall including
a first through hole;
a second through hole; and
a groove to connect ends of the first through hole and the second through hole that open on an outer surface of the bottom wall;

a transparent plate member to cover a portion of the outer surface of the bottom wall including at least the first through hole, the second through hole, and the groove;
a capturing plate disposed on an inner surface of the bottom wall and above the first through hole;

an aspiration tube with one end to be connected to an upper portion of the second through hole and other end to be connected to an aspirating unit, to capture the cell in a micropore of the capturing plate by aspirating;

an injected-cell collecting flow-path to connect a first discharge port for discharging liquid and a well to collect an injected cell in which the substance has been injected, and to guide the injected cell being captured in the micropore in a direction towards the well;

an empty-cell collecting flow-path to connect a second discharge port for discharging liquid and a collection port for collecting an empty cell in which no substance has been injected, and to guide the empty cell near the capturing plate in a direction towards the collection port, which is different from the direction towards the well;

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a pair of additional discharge ports that are provided on both sides of the second discharge port to discharge liquid to a direction parallel to the liquid discharged from the second discharge port before the second discharge port discharges liquid; and

a collection pump provided at one end of the well, to switch aspiration pressure from positive pressure to negative pressure on the injected cell being guided by the injected-cell collecting flow-path,

wherein the first discharge port and the second discharge port alternately discharge liquid.

2. The cell capturing apparatus according to claim 1, wherein the aspirating unit is to aspirate the liquid and the empty cell from the collection port.

3. The cell capturing apparatus according to claim 1, further comprising:

an obstruction member arranged in the empty-cell collecting flow-path between the second discharge port and the capturing surface of the capturing plate and to cause an obstruction in a flow of the liquid.

4. The cell capturing apparatus according to claim 1, wherein the empty-cell collecting flow-path between the second discharge port and the capturing surface of the capturing plate includes a plurality of empty-cell collecting sub-flow-paths.

5. The cell capturing apparatus according to claim 4, wherein the empty-cell collecting sub-flow-paths join together to form a single empty-cell collecting flow-path just before reaching the capturing surface of the capturing plate.

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6. The cell capturing apparatus according to claim 1, further comprising:

a plurality of convex portions arranged in the empty-cell collecting flow-path between the second discharge port and the capturing surface of the capturing plate and causes an obstruction in a flow of the liquid.

7. The cell capturing apparatus according to claim 1, wherein a step is provided around the capturing surface of the capturing plate where the injected-cell collecting flow-path and the empty-cell collecting flow-path respectively intersect.

8. The cell capturing apparatus according to claim 1, wherein an inclination that prevents back flow of injected cells that have flowed into the well is provided at an intermediate position in the flow path that leads from the capturing surface of the capturing plate to the well on the downstream side of the injected-cell collecting flow-path.

9. The cell capturing apparatus according to claim 1, the capturing plate includes at least one capturing through hole for capturing the cell, wherein

the cell capturing plate is disposed on the inner surface of the bottom wall in such a manner that the transparent plate member is located near the capturing through hole.

10. The cell capturing apparatus according to claim 1, wherein the capturing plate is disposed on the inner surface of the bottom wall in such a manner that the capturing plate makes an angle of about 45 degrees relative to a direction of the liquid flow along the empty-cell collecting flow-path.

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